THE NEW YORK ACADEMY OF SCIENCES

(Lyceum of Natural History, 1817-1876)

These Sections and the Division hold meetings regularly, one evening each month, during the academic year, October to May, inclusive.
Two-day conferences also are held at irregular intervals. All meetings are held at the building of The New York Academy of Sciences, 2 East Sixty-third Street, New York 21, New York.

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 50, Art 10 Pages 1209-1404
September 6, 1950

Editor
ROY WALDO MINER

Associate Editor
B J HENEGAN

MEDICAL MYCOLOGY*

Consulting Editor and Conference Chairman
FREDERICK REISS

CONTENTS

PAGE

* This series of papers is the result of a Conference on *Medical Mycology* held by the Section of Biology of The New York Academy of Sciences October 31 and November 1 1947
Publication made possible by grants-in-aid from Wallace & Tiernan Products Inc, Hoffmann LaRoche Inc, Dome Chemicals, Inc, and The Welcome Research Laboratories and by a contribution from the General Funds of The New York Academy of Sciences

INTRODUCTION

By Frederick Reiss
New York University Bellevue Medical Center, New York, N. Y.

It is a pleasant duty and a great honor to introduce this monograph, which is the result of the first conference on medical mycology in the United States. The many authors and the diversity of their topics are indicative of the growing interest in the subject. The purpose of this monograph is not only to present recent data, but also to discuss some of the fundamental aspects of our problems which await elucidation and solution in the future.

Mycology had an earlier start than bacteriology. It continued to attract attention until the 'Seventies or 'Eighties of the last century, when the epoch making discoveries of Pasteur and Koch in the field of infectious diseases brought bacteriology to the fore and relegated mycology to the background, where it has remained for many years. There is little doubt, on the other hand, that mycology gained an important place not only in botany and cognate technology, but also in animal and human pathology.

Though fatalities due to fungus diseases in man are not so common as those due to bacterial infections, nevertheless these mycotic diseases are important. This was recognized and emphasized by the leading medical authorities of our armed forces, in which the incidence of "athlete's foot" assumed high proportions during the last war. At Fort Benning, for instance, fungi were reported to be present in 42 to 64 per cent of the scrapings from feet. The aggravation of pre-existing fungus diseases of the skin in tropical climates was another factor which made us mindful of the importance of medical mycology. The recent epidemic of ringworm infection in scalps of school children, which had its onset during the war and is still not declining, serves as another good example of the public health implications of mycoses. While the number of deep or fatal mycoses is relatively low (in 1942, of 1,385,187 deaths, 359 were caused by fungi in the U. S.), they equal more than half the deaths caused by the whole enteric group, tetanus, and infantile paralysis combined.

While great progress has been made in the simplification of mycological nomenclature, the classification of fungi, unlike the status in bacteriology, has not reached a satisfactory stage. As a result, the same species of fungi are described *de novo* under various new names adding to the confusion. Also while interesting and fundamental contributions have been made in bacteriological and virulogical immunity, we are still uncertain as to the interpretation of many mycological phenomena of immunity. The keratophilic tendency and affinity for dead tissues of the dermatophytes have been recognized for a long time, but the mode of their enzymatic pathogenic action is not yet entirely clear. It is startling to note the difference in immunologic response between infections caused by fungi and fungus like bacteria (*e.g.*, *Mycobacterium tuberculosis* and *leprae*) and those caused by bacteria which elaborate exotoxins. Despite the fact that one of the first

polysaccharides responsible for immunological changes was isolated from trichophytons and blastomycetes, the mechanisms of immunity are not yet clarified to the same degree as they are in many bacterial infections such as tetanus, diphtheria and botulism In the latter group, the exotoxins are the agents of the disease, and recovery is due to the action of antibodies No similar advance has yet been made in mycology The treatment of superficial mycoses with fatty acids signifies a considerable step forward,

tion of this problem

Neither the mode of transmission of the superficial mycoses nor their prevention is fully understood, whereas the prevention of plant mycoses has been suitably accomplished Prophylactic vaccination, if perfected, would be just as desirable against fungus diseases as it is against diphtheria, tetanus, and smallpox

While little difficulty is encountered in the diagnosis of deep mycoses, we are still lagging behind in the cure of most of these diseases Penicillin appears to be a valuable drug in the treatment of actinomycosis, just as

culosis Only in recent years has it been recognized that pulmonary cal cification may be the result of infections other than tuberculosis, namely

the transmission of histoplasmosis, nor has an acceptable explanation been offered as to why certain apparently healthy individuals react strongly to histoplasmin The cross-reaction to blastomycin also awaits further clarification

These are only a few of the many biological and therapeutic complexities with which we are confronted and which provide an impetus and challenge for further investigations I am fully convinced that we are entering a All those concerned must recog n this country in mycological re- d to even greater advances This rpose if it provides a stimulus to new ideas in the solution of many problems

I wish to express my gratitude to The New York Academy of Sciences, and particularly to its able Executive Director, Mrs Eunice Thomas Miner, for having organized this program so efficiently and for making publication of this monograph possible I wish also to thank all the partı cipants for their kind support, which is the augury of success

MYCOLOGICAL RESEARCH AND THE PROGRESS OF MEDICINE

By Carroll W. Dodge

Henry Shaw School of Botany, Washington University, and Missouri Botanical Garden, St Louis, Mo

The relation of certain fungi to human disease was early recognized, especially in the period from 1839 to 1853 in the work of Schoenlein and of Gruby, culminating in Robin's (1853) survey of fungi then associated with human disease This early period was followed by a period of little activity, while the medical profession turned their attention to bacterial diseases Activity was renewed about 1890, when Rénon (1897) thoroughly investigated aspergillosis About the same time, Sabouraud began his work on the dermatophytes, which culminated in his exhaustive treatise in 1919 American work began a few years later on coccidioidal granuloma and blastomycosis (1892–1910), with renewed activity after 1930 The foundation of our knowledge of sporotrichosis was laid by de Beurman and Gougerot (1912) Actinomycosis, maduromycosis, and chromomycosis were outlined between 1890 and 1920, although much important work has been done since Castellani began to study the imperfect yeasts about 1905, developing his taxonomy on biochemical activity Langeron and his students placed their classification upon a much sounder basis of morphology in the 1920's, culminating in the taxonomic works of Stelling Dekker (1931), Lodder (1934) Diddens and Lodder (1942), and Dodge (1935) Grigorakis and Moore had carried out extensive cytological investigations in the decade 1925–1935

By 1935, the principal fungi associated with human disease had been described Since that time, we have largely been filling in details rather neglecting the morphology and cytology of the organisms and turning our attention to pathology and therapeusis and to the nutrition of the fungi The importance of other groups of fungi as allergens has been studied increasingly in the last quarter century

Research in other fields of mycology so far has had little influence on the medical field, but perhaps, in passing, I may mention lines of investigation, still in their early stages, which later may influence our thought Baker (1944) has provided cytologic evidence for heterocaryosis in the imperfect fungi and a cytologic basis for inheritance in the absence of the usual type of the sexual act The current studies of Lindegren and co-workers on the cytology and genetics of yeasts may give us a better idea of stable and variable characters for a better system of classification, while his work on adaptive enzymes may give a better appreciation of enzyme activity and help explain the anomalies which every worker encounters in the study of fermentation Such studies may eventually help us to clear up the confusion created by Castellani in describing only the biochemical characters of so many imperfect yeasts

In looking forward to fields for further research, in many cases we find

two or more organisms isolated from different cases of a clinically similar disease We need better clinical description and differentiation correlated with a careful study of the organism isolated in each case, before we can assume that the same clinical entity is produced by several different fungi A notable example of the work already done in this field is that of the clinical forms of South American blastomycosis where in the early days two distinct clinical forms were treated as a single entity without a careful study of the organisms When a careful correlation of clinical types and fungi isolated was made each organism was found to be associated with a distinct clinical type The field of therapeusis and possible development of antibiotics will only be mentioned as it will be treated much more fully in later papers

We need more appreciation of the relations of vitamin nutrition and the activity of the endocrine systems of the host, especially in the field of dermatology Although it has been long recognized that changes in the endocrine system at puberty produce corresponding changes in the skin (Greenwood and Swartz outlined such a study to me in private conversations a quarter of a century ago) it was fully twenty years before research along these lines entirely independently showed changes in the oil secretion and developed promising new treatments with fatty acids Such research is still in an early stage and we can expect important developments along these lines

While in Guatemala in 1941–1942, I had an opportunity to observe many cases of a partial achromia mostly on the exposed portions (face neck and forearms and sometimes also on the legs) of prepubertal males Quite regularly from the very slight scaling over the partially depigmented areas, I isolated *Hormodendrum fontoynonti* Langeron or a very closely related species reported by Fontoynont and Carougeau (1922) from a similar disease hodi potsy in Madagascar Occasionally I also isolated the same organism from scales not accompanied by the partial achromia and I was inclined to consider the *Hormodendrum* a skin saprophyte Shortly before I left Guatemala a medical friend treated his son for an undescended testis For some months I had observed in the son a very characteristic case of the partial achromia Within two weeks after the treatment, scaling disappeared and the pigmentation became wholly normal After this observation the father and I planned to study and treat selected cases in the school clinics to see whether there was a definite relation between the partial achromia and the gonadal hormones Unfortunately it was at the height of the submarine activity in the Caribbean which had completely interrupted commerce and the War Department was utilizing the total freight capacity of the airlines so there was practically no supply of the hormones in the country I had to return shortly afterward and the matter was dropped without our arriving at any satisfactory conclusion

While in Guatemala I also had the good fortune to study several cases of a disease closely resembling if not identical with tokelau in a small area along the road between Lake Atitlan and Patulul This disease had already been studied by Herrera Solis (1932) and a recent single case was reported by Figueroa and Conant (1940) Frequently *Endodermophyton*

has been isolated from the characteristic scales of these patients The scales from the patients were carefully collected in sterile petri dishes and blood samples were taken for Wassermann reactions as some of Herrera Solis s patients had been Wassermann positive All samples were Wassermann negative ruling out any possible syphilides Microscopic examination of the scales showed fungus hyphae of the ring worm type

From twelve cases, I isolated *Endodermophyton* in two cases *Epidermophyton* in two cases *Ectotrichophyton* in only four cases In two prepubertal boys I isolated only imperfect yeasts Two other cases not so clinically typical yielded *Ectotrichophyton* One of the *Endodermophyton* cases also yielded a colony of an *Ectotrichophyton*

On reviewing my clinical notes I found that all the patients except one prepubertal boy were conspicuously undernourished Herrera Solis had noted the same condition in his eight cases Normals examined in the same small community were well nourished as is general in that part of Guatemala Since vitamin A seems to affect the condition of the skin more than the other vitamins it seems likely that the lack of this vitamin had so altered the outer horny layer of the skin that the *Endodermophyton* and other skin saprophytes had been able to grow more luxuriantly than on normal skin and had contributed to the characteristic scaling While I observed only one case of obvious endocrine dysfunction (an elderly woman with a large goiter) in these patients it may have been a complicating factor as varying degrees of thyroid dysfunction are said to be common in this area

We should also recognize the possibility in fungus infections of a symbiosis of two or more organisms perhaps complicated by endocrine dysfunction especially at the thyroid as seems to be the case in seborrhea and acne

Another question has intrigued me for some time Why are some organisms more invasive in some individuals than in others? As an example I may cite two successive cases of tinea cruris observed in Guatemala The first was a sailor nervous from the submarine sinkings he had witnessed in the Caribbean during the previous few weeks The lesions which had developed in about a week occupied the gluteal fold nearly covered the buttocks the perineum, the inner aspects of the thighs half way to the knees and followed the pubic hair tract nearly to the umbilicus The pruritus was intense and he was continually walking the floor of his room He showed considerable self-control however as there was little excoriation and no secondary infection He was a blond with very fair skin and little body hair The next case was a placid Guatemalan business man The lesion which had been developing for about a month was a typical round plaque about 6 cm in diameter on the inner aspect of the thigh opposite the scrotum The patient did not complain of pruritus although there was some excoriation He was a brunet with a darker skin than the first patient but much lighter than the average Guatemalan The body hair was abundant over the trunk and thighs Both seemed equally well nourished without endocrine dysfunction Physical factors of the environment were essentially similar as the Guatemalan s business kept him in the tropical lowlands

most of the time Had the nervous excitement of the sailor so altered the outer layer of the skin that the fungus had found conditions for invasion and growth more favorable?

We need more study of the saprophytic phase in the life cycles of pathogenic fungi Where does the fungus vegetate between human cases? Are there subclinical cases which keep the organism going between the severer, recognized cases? How are the organisms transmitted? These are some of the questions which need prompt solution Yet, except in the case of the dermatophytes we have very little information, and even with the dermatophytes there is much more to be learned

Finally we need more monographic studies, including the saprophytic as well as the parasitic species in the groups concerned, embracing the morphology, cytology, and physiology, especially in the imperfect yeasts and the groups with dark colored mycelium, such as the *Dematium Hormodendrum* series

Bibliography

BAKER, G E 1944 Heterocaryosis in *Penicillium notatum* Bull Torrey Bot Club 71 267 373

sideae]

RÉNON L 1897 Etude sur l aspergillose chez les animaux et chez l homme Masson et Cie Paris

ROBIN C 1853 Histoire naturelle des végétaux parasites qui croissent sur l homme et sur les animaux vivants Atlas Paris

SABOURAUD, R 1910 Maladies du cuir chevelu III Les maladies cryptogamiques Les Teignes Masson et Cie Paris

STELLING DEKKER N M 1931 Die Hefesammlung des Centraal Bureau voor Schimmelcultures Beitrage zur einer Monographie der Hefearten I Die sporogenen Hefen Verhandel K Akad Wetensch Amsterdam Afdeel Natuurk 28(1) 1 547

PUBLIC HEALTH ASPECTS OF FUNGUS INFECTIONS

By S B Salvin

Division of Infectious Diseases National Institutes of Health Bethesda Maryland

Fungus infections in man although less frequent than bacterial are still numerically important For example of the 92 933 deaths due to infections and parasitic diseases in the United States in 1945 284 or 0 3 per cent were due to mycoses[1] This was approximately equal to the number of deaths reported as caused by scarlet fever measles or the typhuslike diseases (due to rickettsia) and was more than the total of all deaths recorded from rabies smallpox relapsing fever leprosy brucellosis paratyphoid fever plague cholera and anthrax It should be realized of course that effective control measures are employed against some of the aforementioned diseases whereas control methods against the mycoses not only are not practiced but generally are not even known It should also be borne in mind that the dermatophytoses although characteristically nonfatal are extremely common probably equaling the most widespread of the bacterial or virus diseases in prevalence

Many species of fungi have been characterized as having produced disease in man and the higher animals Some such as *Blastomyces brasiliensis* and *Blastomyces dermatitidis* have been isolated only from the parasitic state Others such as *Aspergillus fumigatus* and *Sporotrichum schencki* have been demonstrated as existing in nature both as pathogens and as saprophytes Indeed it is often difficult in some instances to determine whether the role the fungus plays within a given host involves a parasitic or saprophytic existence

The association of a potentially pathogenic fungus with man does not necessarily imply that parasitism exists The frequent isolation of *Candida albicans* from the intestinal tract of cases of sprue precipitated the erroneous belief that that fungus was the etiologic agent for the disease Similarly the frequent association of various species of *Aspergillus Penicillium etc* with pinta pointed toward these fungi as the causative organisms Repeated demonstration of the fungus in the host and its isolation therefrom to be followed by the development of disease in experimentally infected animals and by the subsequent recovery of the causative fungus are often necessary to prove the pathogenic role of an organism

The presence of these sometime pathogens in pus skin lesions or sputum should be weighed very carefully before they are assigned etiological significance For example *Candida albicans* is often isolated from sputum especially if the sputum is permitted to remain at room temperature for several hours under which circumstance rapid multiplication of the fungus occurs Thus examination of old sputum may produce an erroneous picture of the true mycologic flora Even the isolation of this species from fresh sputum does not warrant a diagnosis of moniliasis for Benham and Hopkins[2] found the fungus on 6 per cent of normal tongues Todd[3] in the throat and mouth of 14 per cent of 1000 normal persons and Knighton[4] in 24 per cent of 146

normal mouths No correlation was found between the presence of *C albicans* and dental caries or diseases of the gums Up to date no practical method has been presented for evaluating properly this occasionally pathogenic, but often apparently noninjurious, fungus in sputum from unidentified pulmonary disease

There are four deep mycoses of public health interest which will be discussed here in some detail, with emphasis on their geographic distribution, their natural habitat, their mode of transmission, and their mode of infection The first is actinomycosis, the common type of which, caused by the anaerobic *Actinomyces bovis*, is world wide in distribution and doubtless is the most common of the deep mycoses

using aerobic methods for cultivation, isolated an aerobic species, probably a *Streptomyces*, which he erroneously described as *A bovis* This isolate

isolated from outside the human or animal body, and probably, do not thrive ordinarily in the outside world because of their inability to grow at low temperatures their need for reduced oxygen tension and their lack of spores

The claim that Bostroem's organism caused actinomycosis was partly responsible for the belief that actinomycosis is primarily a disease of rural groups transmitted either by contact with infected animals or by traumatization with infected grasses straws, or grains Davis[9] examined 46 new cases with regard to this belief, and found that, in general, rural workers were no more susceptible to infection than urban individuals Of the 46 cases only 15 were farmers with but 3 having a history of chewing grasses In addition no definite instance has been recorded where the

sponsible for this condition, true actinomycetes were also present Nevertheless no case of actinomycosis in man has been demonstrated as resulting from the ingestion of such infected food Baracz[11] and McKenty[12] reported cases of possible transmission of the disease from man to man, but the evidence is not very convincing

The early studies of Wright[13] and Lord[14, 15] demonstrated that *A bovis* may be found in carious teeth Lord also isolated the organism from tonsillar crypts of patients without actinomycosis, work later confirmed by Emmons[16] and by Slack[17] The potentially pathogenic *A bovis* may thus occur in small numbers as part of the flora of the normal mouth and, together with the lactobacilli and the oral spirochetes, make the oral and pharyngeal mucous membranes its natural habitat

The method whereby this fungus becomes transformed into a destructive parasite in adjacent or remote tissues is still a matter of conjecture Local trauma, although possibly contributory, is not the main causative factor, as indicated by the relative infrequency of the infection in man It is interesting to note, however, that a history of tooth extraction or an injury to the mouth has been recorded in cervico facial actinomycosis,[18] and that instances of actinomycosis in the hand have followed punctures of the

ease about the jaws and neck, the root canal of the tooth being the channel of infection[22]

Finally,

such calcul

omycosis

supragingival and subgingival calculus and salivary duct stones—always contain a stroma of *Actinomyces* and *Leptotrichia* vegetations, with typical actinomycotic clubs occasionally demonstrable He isolated anaerobic strains repeatedly from the calculi, and succeeded in producing concretions *in vitro* with pure cultures The theory has therefore been proposed that calculus is produced by the activity of *Actinomyces* and to some extent by *Leptotrichia*, both of which induce the precipitation of salts from the

that fragments of such calculus may become detached from the teeth, and on becoming impacted in tissue, initiate progressive actinomycosis Although there is no direct evidence for this view, it is logical, especially since Slack[17] and Rosebury *et al*[26] succeeded in producing progressive actinomycosis experimentally in animals with strains of *A bovis* isolated from

involved therein, is sporotrichosis, first recognized by Schenck[27] in this country in 1898, and shortly afterwards by De Beurmann in France[28] It has since been reported from all the continents, although a preponderance of the reported cases stem from France, the United States, and South America There is some indication of an endemic tendency, since five sixths of the 57 cases cited by Ruediger[29] were from the Missouri Valley, and 130 out of the 148 cited by Foerster[30] were from the Mississippi River basin, primarily, certain localities in the Dakotas, Nebraska Wisconsin, Kansas, and Missouri This distribution may suggest a preference of the fungus for certain types of vegetation and for particular climatic conditions

Of 18 cases described by Foerster,[30] 14 were employees of a tree nursery, and at least 10 undoubtedly acquired the infection by traumatization with the thorns of the barberry shrub, primarily the Japanese barberry, *Berberis*

thunbergii In two of these cases, barberry thorns were removed from the primary lesions after suppuration had started, in 6, deeply embedded barberry thorns had been removed from a few days to two weeks prior to the

the barberry seemed to be the source of infection Wakefield observed

florists The fungus was also obtained from timbers of a mine in South Africa, and from the miners exposed to this material[32] These and the

to convey the disease to humans

Benham and Kesten[33] described the saprophytic growth of the fungus on experimentally inoculated barberry thorns and in carnation buds, and showed that the fungus retained its virulence for animals after a brief saprophytic life on plants

Sporotrichosis has also been reported in lower animals,[34] appearing spontaneously in horses and livestock in certain parts of the United States However, the relative absence of the disease among the veterinarians and workers in the areas where equine sporotrichosis is common indicates the unimportance of this channel of contact Ruediger[29] reported one

The third mycosis of interest here is coccidioidomycosis Originally, this disease was believed to be limited to the Chaco region of Argentina[41, 42] and to the San Joaquin Valley in California[43] but recent work by Phillips,[44] Smith,[45] and others[46–50] has shown the fungus to be present throughout

uthern Utah, Arizona, New

ently assumed added signif-

who were stationed in the

through Each individual

in these groups, on developing an acute respiratory infection, becomes another possible case of coccidioidomycosis with its added diagnostic and therapeutic problems

The infection by *Coccidioides immitis* is evidently not transmitted directly from one individual to the next, thus, there is no reason for isolation of the patient The parasitic or spherule phase of the fungus which appears in human sputum or pus is infectious when introduced experimentally into laboratory animals,[51] but seemingly is of no importance in the natural direct transmission of the ailment The chlamydospores from the saprophytic

phase of the fungus are the main infectious agents Epidemiological studies, the clinical picture, the pathology of disseminated infections, accidental

be present in the dust and the fungus to be thriving in the soil from which the dust arose

Little success has been evident, however, in attempts to isolate *Coccidioides* from the soil The fungus was isolated first from the soil near

Hundreds of other attempts to isolate *Coccidioides* from the soil of endemic areas have failed.

Recent studies however, have presented the possibility of a rodent reservoir of *Coccidioides* Of 303 animals from an endemic area, trapped and examined by Emmons[35] for possible pathogenic fungi, 128 were infected: 16 with *Coccidioides immitis* alone, 9 with both *C immitis* and *Haplosporangium parvum*, and 92 with only *H parvum* The types of animals

nized as an agent in human disease

Coccidioidomycosis in the rodents apparently was a chronic disease which did not seem to interfere with the normal reproduction and development of the animals It was suggested that the fungus may be primarily a pathogen of rodents and present in soil that has been contaminated by infected rodents

edge of the disease Those who have had primary coccidioidomycosis,

reactions to coccidioidin in people living within the endemic areas of coccidioidomycosis, and virtually no reactions in other nonresident groups They concluded that some of these calcifications may be due to *Coccidioides*

C E Smith[56] reported that the first symptoms appeared 7 to 21 days after inhalation of the chlamydospores, and sensitivity to coccidioidin in 10 to 45 days His survey of the San Joaquin Valley cases over a period

thunbergii In two of these cases, barberry thorns were removed from the primary lesions after suppuration had started, in 6, deeply embedded barberry thorns had been removed from a few days to two weeks prior to the

the barberry seemed to be the source of infection Wakefield observed

florists The fungus was also obtained from timbers of a mine in South Africa, and from the miners exposed to this material [32] These and the preponderance of cases among rural inhabitants and others close to the soil suggest that the favorite habitats of *Sporotrichum schencki* are probably shrubs, grasses, and the like, although bushes with thorns are more apt to convey the disease to humans

Benham and Kesten[33] described the saprophytic growth of the fungus on experimentally inoculated barberry thorns and in carnation buds, and showed that the fungus retained its virulence for animals after a brief saprophytic life on plants

Sporotrichosis has also been reported in lower animals,[34] appearing spontaneously in horses and livestock in certain parts of the United States However, the relative absence of the disease among the veterinarians and workers in the areas where equine sporotrichosis is common indicates the unimportance of this channel of contact Ruediger[19] reported one case in which the initial lesion of the disease appeared on the site of a recent hen bite, and another in which the first nodule developed on the site of a wound from a wire in a barn containing a sporotrichotic horse The disease has been described as occurring spontaneously in dogs rats rabbits, cats, and other small mammals [35–40]

The third mycosis of interest here is coccidioidomycosis Originally, this disease was believed to be limited to the Chaco region of Argentina[41, 42] and to the San Joaquin Valley in California [43] but recent work by Phillips,[44] Smith [45] and others[46–50] has shown the fungus to be present throughout the arid Southwest, in southern California, southern Utah, Arizona, New Mexico and west Texas This mycosis has recently assumed added significance because of the large number of soldiers who were stationed in the endemic area and the many tourists who pass through Each individual in these groups on developing an acute respiratory infection, becomes another possible case of coccidioidomycosis with its added diagnostic and therapeutic problems

The infection by *Coccidioides immitis* is evidently not transmitted directly from one individual to the next, thus there is no reason for isolation of the patient The parasitic or spherule phase of the fungus which appears in human sputum or pus is infectious when introduced experimentally into laboratory animals [51] but seemingly is of no importance in the natural direct transmission of the ailment The chlamydospores from the saprophytic

phase of the fungus are the main infectious agents Epidemiological studies, the clinical picture, the pathology of disseminated infections, accidental laboratory infections, and experimental production of the disease in guinea pigs by inhalation point toward the probability of infection by inhalation of air borne spores There is a good correlation of exposures to dust storms and onset of symptoms, with the result that the spores were assumed to be present in the dust and the fungus to be thriving in the soil from which the dust arose.

Little success has been evident, however, in attempts to isolate *Coccidioides* from the soil The fungus was isolated first from the soil near a Delano, California, ranch house where there were four cases of coccidioidal granuloma,[52] then in Panoche Valley, California, near a burrow from which a group had dug a rattlesnake,[53] and, thirdly, from five (out of 150) soil samples collected on the desert near the village of San Carlos, Arizona[54] Hundreds of other attempts to isolate *Coccidioides* from the soil of endemic areas have failed

Recent studies, however, have presented the possibility of a rodent reservoir of *Coccidioides* Of 303 animals from an endemic area, trapped and examined by Emmons[55] for possible pathogenic fungi, 128 were infected: 16 with *Coccidioides immitis* alone, 9 with both *C immitis* and *Haplosporangium parvum*, and 92 with only *H parvum* The types of animals found infected were the pocket mouse, *Perognathus*, the grasshopper mouse, *Onychomys*, the kangaroo rat, *Dipodomys*, and a ground squirrel, *Citellus*, with the pocket mouse (*Perognathus*) and the kangaroo rat (*Dipodomys*) showing the highest percentages of natural infection, namely, 15 per cent and 17 per cent, respectively This second fungus, *Haplosporangium parvum*, which, in some respects, resembles *Coccidioides* in the parasitic phase, causes a somewhat similar pulmonary disease in rodents, but has not yet been recognized as an agent in human disease

Coccidioidomycosis in the rodents apparently was a chronic disease which did not seem to interfere with the normal reproduction and development of the animals It was suggested that the fungus may be primarily a pathogen of rodents and present in soil that has been contaminated by infected rodents

The diagnosis of coccidioidomycosis in humans depends upon the demonstration of the fungus in pus, sputum, or tissues The coccidioidin skin tests and serological tests have assisted greatly, however, in adding to the knowledge of the disease Those who have had primary coccidioidomycosis, even in clinically inapparent form, retain a skin sensitivity for many years

Aronson, Saylor and Parr,[56] in a study of calcified pulmonary nodules in tuberculin negative individuals, found a very high percentage of positive reactions to coccidioidin in people living within the endemic areas of coccidioidomycosis, and virtually no reactions in other nonresident groups They concluded that some of these calcifications may be due to *Coccidioides*

C E Smith[46] reported that the first symptoms appeared 7 to 21 days after inhalation of the chlamydospores and sensitivity to coccidioidin in 10 to 45 days His survey of the San Joaquin Valley cases over a period

of 17 months illustrated the association between the onset of disease, field work by the inhabitants, and the presence of dust

The seasonal variation of the disease and its association with dust were ... *et al*[57] at four Army airfields ... In the fall of 1941, when ... there were extensive scarred surfaces and much dust That season of maximal dust provided the highest coccidioidal rates of the study The following year, lawns were planted, roads were paved, airstrips hard surfaced, and the incidence of coccidioidal infections was cut in half When the fields were oiled in June, 1944, the incidence was lowest in their history

Finally, among the deep mycoses, we come to histoplasmosis, a disease originally described in Panama in 1906,[58] [59] but since then reported from the United States,[60] South America,[61] Java,[62] England[63] Philippine Islands,[64] Germany,[65] Turkey,[66] and several other areas[67] The disease is sporadic ...

therefore may be possible reservoirs of the disease

Although only slightly more than one hundred cases have been reported ...

Palmer,[70] for example, after a study of 3000 student nurses, concluded "(a) that mild, probably subclinical infection with *H capsulatum* (or an immunologically related organism) is widely prevalent in certain states and relatively infrequent in others (b) that, in general, those states in ...

although the exact causes of nontuberculous pulmonary calcification still are in doubt Proof that histoplasmosis is one of the etiologic agents of this condition awaits the use of a specific diagnostic procedure and the isolation of *Histoplasma* from the hypothecated frequent mild cases of the disease

Probably one of the most troublesome public health problems of the past few years was ringworm of t ... *audouini* M

canis and several species of *Trichophyton*. Several epidemic outbreaks of inea capitis in large cities chiefly in the eastern parts of this country, have een recorded

Montgomery in a discussion after the paper of Lewis *et al* [85] reported an average of 77 cases of tinea capitis per year from 1935 to 1942 at a New York City clinic with 47 4 per cent caused by *M audouini* In 1943 at the same clinic the total number of cases increased greatly to 572, with 86 7 per cent caused by *M audouini* This increase in the percentage of cases caused by *M audouini* and its appearance in epidemic proportions probably were partly the cause of its being more contagious more resistant to treatment and better adapted for human infection than *M canis* Benedek and Felscher[86] reported 140 cases in Chicago from 1940 to 1942 of which 81 5 per cent were caused by *M audouini* and 12 2 per cent by *M canis* Livingood and Pillsbury[87] reported 130 cases in Philadelphia in 1941 of which 96 2 per cent were caused by *M audouini* Miller Lowenfish and Beattie[88] reported 928 new cases from 1943 to 1945 of which 96 9 per cent were due to *M audouini* Schwartz *et al* [89] reported 565 out of 8 657 children infected in their survey at Hagerstown, Maryland with all but 8 caused by *M audouini* Carrick [90] in a survey of 3565 Detroit elementary school children chosen at random found 2 7 per cent with signs of infection as indicated by Wood s light On the basis of the total number of children subject to ringworm of the scalp he estimated that there were about 6000 cases of tinea capitis in the Detroit public schools Lewis *et al* [85] believed that these recent outbreaks resulted primarily from the decreased maternal care and supervision during the war the movement of infected children from place to place because of reasons connected with the war effort the crowding of institutions for children and the concomitant inefficient supervision

Among the more important steps for prevention and control are the early recognition and reporting of the disease Isolation and early adequate treatment are necessary to prevent spread to other children and to other areas of the body of the same individual Most dermatologists believe that infected children should be excluded from school until recovery and in institutions the infected should be separated from the healthy Each infected child should be provided with a stocking cap or some similar head covering that can be burned after use All contacts with infected children should be examined with Wood s light a piece of apparatus that should be available at the local school or health department Educational programs should be pursued for parents in epidemic areas

Schwartz *et al* [89] in their studies at Hagerstown found 12 1 per cent of the boys infected and only 2 1 per cent of the girls Apparently the boys and girls had equal exposures in schools playgrounds homes and movies but not in the barber shops which were attended by nearly all the boys but by only a few girls Examination of the barber shops revealed generally poor sanitary conditions with infected hairs found frequently in the electric clippers Of the infected boys 65 per cent had the fungus present in the clipper area only 31 per cent over the head and 4 per cent on the crown

of the head only In the girls, the fungus was present always in the area of the hair part

According to Lee,[91] the results of a questionnaire to state health departments in August, 1946 showed that ringworm of the scalp was a reportable disease in three states only, Illinois, Ohio, and Pennsylvania The city health departments of St Louis, Cleveland, and Philadelphia, however, required the reporting of this infection In Jersey City and Newark, tinea capitis is categorized as a public health problem, but reporting the disease is *not required by law*

The most common of all the fungus diseases are the dermatophytoses, caused by ***Epidermophyton floccosum*** and by various species of ***Trichophyton*** Among these the incidence of dermatophytosis of the foot is probably the highest For example, Legge, Bonar, and Templeton[92] found that 51 5 per cent of the men admitted to the University of California during a single year had athlete's foot," and that the percentage increased to 78 6 by the end of the year Alderson and Reich[93] reported that dermatophytosis of the foot represented 24 7 per cent of the skin diseases in a student health service Lomholt[94] reported that the incidence by clinical diagnosis in school children in Copenhagen was almost 50 per cent Of 354 men surveyed in an industrial plant,[95] 36 4 per cent were found by cultural examination to be infected

Many compounds and methods have been used in the attempt to control these skin pathogens One of the more common ones involved the use of 1 per cent sodium hypochlorite in foot baths, in spite of the fact that Bonar and Dreyer[96] found the fungi in skin scales still alive after one hour's exposure to the hypochlorite Dermatophytes were found to flourish on old

standard power laundry techniques kill the dermatophytes, primarily because of the high temperatures used, although standard dry-cleaning sol-

were inflammatory disorders of the peripheral vascular system, the lesions of which are characterized by endothelial necrosis and inflammation, thrombosis, and by painful ulcerations or gangrene of extremities These lesions he considered as possibly being aroused by the inflammatory action of the products of some of the dermatophytes If true, this role should increase still further the importance of the dermatophytes as a public health problem

Bibliography

1 Vital Statistics—Special Reports National Summaries 27(2) May 6 1947
2 BENHAM R W & A M HOPKINS 1933 Yeastlike fungi found on the skin and in the intestines of normal subjects Arch Derm & Syph 28 532

3 Todd, R L & W W Hermann 1937 Studies on yeast like organisms isolated from the mouths and throats of normal persons. Amer Jour Hyg 25 212
4 Knighton, H T 1939 A study of *Monilia* and other yeastlike organisms found in the oral cavity J Dent Res 18 103
5 Bollinger O 1877 Ueber eine neue Pilzkrankheit beim Rinde Centralbl Med Wissen 15 481
6 Harz C O 1879 *Actinomyces bovis* ein neuer Schimmel in den Geweben des Rindes Deutsche Zeitschr f Tiermed Suppl to Bd 5 (Jahresber d k Central Tierarznei Schule in München f 1877–78 125)
7 Wolff, M & J Israel 1891 Ueber Reinkulturen des Actinomyces und seine Uebertragbarkeit auf Thiere Arch Path Anat Physiol (Virchow) 126 11
8 Bostroem E 1891 Untersuchungen uber die Aktinomykose des Menschen Beitr Path Anat Allg Path 9 1
9 Davis M I J 1941 Analysis of forty six cases of actinomycosis with special reference to its etiology Am J Surg 52 447
10 Griffith F 1916 On the pathology of bovine actinomycosis J Hyg 15 195
11 Baracz R V 1889 Ubertragbarkeit der Aktinomykose von Menschen auf den Menschen Wein med Presse 30 6
12 McKenty F E 1913 A study of cases of actinomycosis Am J Med Sci 145 835
13 Wright J H 1905 The biology of the microorganisms of actinomycosis J Med Res 8 349
14 Lord F T 1910 The etiology of actinomycosis The presence of Actinomyces in the contents of carious teeth and the tonsillar crypts of patients without actinomycosis J A M A 55 1261
15 Lord F T 1910 A contribution to the etiology of actinomycosis The experimental production of actinomycosis in guinea pigs inoculated with the contents of carious teeth Boston Med & Surg J 163 82
16 Emmons C W 1938 The isolation of *Actinomyces bovis* from tonsillar granules Pub Health Rep 53 1967
17 Slack J 1942 The source of infection in actinomycosis J Bact 43 193
18 Cope V Z 1938 Actinomycosis Oxford University Press London
19 Cope V Z 1915 A clinical study of actinomycosis with illustrative cases Brit J Surg 3 55
20 McWilliams C A 1917 Actinomycosis of phalanx of finger Ann Surg 66 117
21 Robinson R A 1944 Actinomycosis of the subcutaneous tissues of the forearm secondary to a human bite J A M A 124 1049
22 Lord F T & L D Trevett 1936 The pathogenesis of actinomycosis J Inf Dis 58 115
23 Naeslund C 1925 Studies of Actinomycetes from the oral cavity Acta Path Microbiol Scand 2 110
24 Naeslund C 1926 Studies of tartar formation Acta Path Microbiol Scand 3 637
25 Colebrook L 1931 The anaerobic Actinomyces A system of bacteriology in relation to medicine Med Res Council London 8 78
26 Rosebury T L J Epps & A R Clark 1944 A study of the isolation cultivation and pathogenicity of *Actinomyces israeli* recovered from the human mouth and from actinomycosis in man J Inf Dis 74 131
27 Schenck B R 1898 On refractory subcutaneous abscesses caused by a fungus possibly related to the Sporotricha Bull Johns Hopkins Hosp 9 286
28 de Beurmann L & H Gougerot 1912 Les Sporotrichoses Felix Alcan Paris
29 Ruediger, G F 1912 Sporotrichosis in the United States J Inf Dis 11 193
30 Foerster H R 1926 Sporotrichosis, an occupational dermatosis J A M A 87 1605
31 Emmons C W 1946 Personal communications
32 du Toit C J 1942 Sporotrichosis on the Witwatersrand Proc Transvaal Mine Med Officers' Assoc 22 111
33 Benham R W & B Kesten 1932 Sporotrichosis its transmission to plants and animals J Inf Dis 50 437
34 Meyer K F 1915 The relation of animal to human sporotrichosis J A M A 65 579
35 Page, C G L Frothingham & J B Paige 1910 Sporothrix and epizootic lymphangitis J Med Res 23 137

36 GOUGEROT & CARAVEN 1908 Sporotrichose spontanée du chien Presse Med 16 337
37 ... bei Menschen und Ratten beobach
J A M A 55 2213
ose des animaux Cong internat
40 OLSON G M 1912 A case of sporotrichosis in North Dakota probable infection from gophers J A M A 59 911
41 POSA
d
42 WER
43 RIXFORD E 1894 A case of protozoic dermatitis Occident Med Times 8 704
44 PHILLIPS E W 1939 Presence of coccidioidal infection in Phoenix Southwestern Med 23 48
45 SMITH L M 1933 Coccidioidal granuloma in western Texas Arch Derm & Syph. 28 175
46 RIXFORD E E C DICKSON & M D BECK 1931 Coccidioidal granuloma
Coccidioides fungus
rt Kern Co Dept
mmitis intradermal
tern Med 48 87
op Med 19 199
nship of coccid oi
occidio domycosis
52 STEWART R A & K F MEYER 1932 Isolation of *coccidioides immitis* (Stiles) from soil Proc Soc Exp Biol & Med 29 937
53 SMITH C E & ...
infection We
54 EMMONS C W
Health Rep
55 EMMONS C W & L L ASHBURN 1942 The isolation of *Haplosporangium parvum* n sp and *Coccidioides immitis* from wild rodents Pub Health Rep 57 1715
56 S
57 S
58 D
46 1255
59 DARLING S T 1908 Histoplasmosis a fatal infectious disease resembling kala azar found among the natives of tropical America Arch Int Med 2 107
60 HANSMANN G H & J R SCHENKEN 1934 A unique infection in man caused by a new yeast like organism a pathogenic member of the genus Sepedonium Amer J Path 10 731
61 PARA M 1946 Histoplasmosis in Brazil Amer J Trop Med 26 273
62 MULLER, H 1932 Histoplasmose in Oost Java Geneeskundig Tijdschrift v Nederlandsch Ind e 72 889
63 DERRY D C L W I CARD R WILSON & J T DUNCAN 1942 Histoplasmosis
64 S
65 S
66 SAGLAM T 1946 Histoplasmosis
67 KERVRAN P & R ARETAS 1946 Deux cases d histoplasmose observés au Soudan français Maroc Med J 25 27

68 McLeod, J H C W Emmons S Ross, & F G Burke 1946 Histoplasmosis—a report of four cases, two in siblings Histoplasmin test and other diagnostic procedures J Pediat 28 275

69 De Monbreun W A 1939 The dog as a natural host for *Histoplasma capsulatum* Report of a case of histoplasmosis in this animal Amer J Trop Med 19 565

70 Callahan W P 1944 Spontaneous histoplasmosis occurring in a dog Amer J Trop Med 24 363

71 Thuringer, J M 1944 Histoplasmosis report of its occurrence in a dog Arch Path 37 140

72 Birge R F & W H Riser 1945 Canine histoplasmosis Report of two cases N Amer Vet 26 281

73 Tomlinson, W J & R G Grocott 1945 Canine histoplasmosis A pathologic study of the three reported cases and the first case found in the Canal Zone Amer J Clin Path 15 501

74 Seibold H R 1946 A case of histoplasmosis in a dog J Amer Vet Med Assoc 109 209

75 Emmons C W J A Bell, & B J Olson 1947 Naturally occurring histoplasmosis in *Mus musculus* and *Rattus norvegicus* Pub Health Rep 62 1642

76 Christie A & J C Peterson 1945 Pulmonary calcification in negative reactors to tuberculin Am J Pub Health 35 1131

77 Christie A & J C Peterson 1946 Pulmonary calcification and sensitivity to histoplasmin tuberculin and haplosporangin J A M A. 131 658

78 Christie A & J C Peterson 1946 Histoplasmin sensitivity J Pediat 29 417

79 Palmer C E 1945 Nontuberculous pulmonary calcification and sensitivity to histoplasmin Pub Health Rep 60 513

80 Palmer C E 1946 Geographic differences in sensitivity to histoplasmin among student nurses Pub Health Rep 61 475

81 Olson B J, J A Bell & C W Emmons 1947 Studies on histoplasmosis in a rural community Am J Publ Health 37, 441

82 Furcolow, M L R H High & M F Allen 1946 Some epidemiological aspects to histoplasmin and tuberculin Pub Health Rep 61 1132

83 Sontag L W & J E Allen 1947 Lung calcifications and histoplasmin tuberculin skin sensitivity J Pediat 30 657

84 Emmons C W, B J Olson, & W W Eldridge 1945 Studies of the role of fungi in pulmonary disease I Cross reactions of histoplasmin Pub Health Rep 60 1383

85 Lewis G M S H Silvers, A Cipollaro E Muskatblit & H H Mitchell 1944 Measures to prevent and control an epidemic of ringworm of the scalp New York State J Med 44 1327

86 Benedek T & I M Felscher 1944 Epidemiology of tinea capitis Arch Derm & Syph 49 120

87 Livingood C S & D M Pillsbury 1944 Ringworm of the scalp—prolonged observation family investigation cultural and immunologic studies in 130 cases J Invest Derm 4 43

88 Miller J L F P Lowenfish, & S F Beattie 1946 Local treatment of tinea capitis J A M A 132 67

89 Schwartz L S M Peck I Botvinik A L Leibovitz & E S Frazer 1946 Control of ringworm of the scalp among school children in Hagerstown Maryland 1944–45 Pub Health Bull No 294 1

90 Carrick L 1946 The epidemiology of tinea capitis in Detroit school children J Michigan Med Soc 45 347

91 Lee R K C 1947 Epidemic tinea capitis a public health problem Yale J Biol & Med 19 547

92 Legge R T L Bonar & H J Templeton 1933 Epidermomycosis at the University of California Arch Derm & Syph 27 12

93 Alderson, H E & A Reich 1937 Incidence of dermatoses in a student health service Arch Derm & Syph 36 57

94 Lomholt S 1938 Ueber die Fussmykosen in Kopenhagen und ihre Behandlung mit Mykoten Med Klin 34 118

95 Neal, P A. & C W Emmons 1939 Dermatitis and coexisting fungous infections among plate printers Pub Health Bull No 246 1

96 Bonar L & A D Dreyer 1932 Studies on ringworm funguses with reference to public health problems Amer J Pub Health 22 909

97 Berberian, D A 1938 Dermatophytosis of the feet sources and methods of prevention and reinfection Arch Derm & Syph 38 367
98 Ayers, S, N P Anderso
in the control of superfi
99 Henderson, Y 1932
formaldehyde as a mea
100 Thompson K W 1941
eral vascular infections
101 Thompson, K W 1943
tion and gangrene of the

EVALUATION OF CLASSIFICATION OF PATHOGENIC FUNGI

By Morris Moore

The Barnard Free Skin and Cancer Hospital, the Barnes Hospital, and the Department of Dermatology, Washington University School of Medicine, St Louis, Missouri

The necessity for classifying pathogenic fungi is no less urgent or important than in the cases of plant life in general and animal life. The confusion existing in man made attempts at the orderly arrangement in a phyletic scheme of pathogenic fungi, however, has made it essential, more than ever, that such a classification be clearly defined and established.

To evaluate properly the classification of pathogenic fungi, it would be in order to set forth the criteria by which the taxonomist is necessarily governed in arriving at a justifiable classification. Of prime importance is morphology. The recognition of the various forms and cell structures which serve to make up the organism and consequently relegate it to an established genus and species is a chief factor. Much depends on the interpretation of cell forms, especially in the absence of the perfect structure, the result of the sexual act. The size and shape of a specific cell structure, such as the macroconidium or fuseau, is used by some as a means of differentiating the genera *Microsporum*, *Trichophyton*, and *Epidermophyton* (FIGURE 1).[1] Where the perfect form is present, and this is a rarity with pathogenic fungi, the classification of the fungus is relatively simple. Since most of the pathogenic fungi are members of the Fungi Imperfecti, classification depends on morphologic characteristics, including methods of spore formation, types of cell structures, location of spores, and special organs. As important as morphology is, however, variations may result from alteration of environmental or physical phenomena and consequently must be viewed and interpreted with caution.

Gross cultural characteristics, like microscopic morphology, play an important role in diagnostic procedure. Size, shape, color, texture, and surface markings are of value in the macroscopic determination of fungi. As in the case of microscopic morphology, gross cultural characteristics may also be altered perceptibly by external factors, which should be considered by the investigator.

In a study of pathogenic fungi with classification in view, efforts are directed towards a determination, if possible, of the perfect stage, the sexual act, and the resulting structure. To this end, the fungus is grown on a medium which might prove to be suitable for such a determination. Altered physiologic response of the fungi due to environmental factors plays important roles in such a procedure.

What are some of these factors? To name a few, we have various nutritional substances, both organic and inorganic, temperature, oxygen requirements, hydrogen ion concentration, moisture, liquid or solid mediums, various physical factors such as visible light, infra red rays, Grenz rays, Roentgen rays (X rays), radium emanations, and perhaps radioactive isotopes. To these must be added genetic factors such as may be observed in cytologic studies and in single spore cultures and which may also be altered

EVALUATION OF CLASSIFICATION OF PATHOGENIC FUNGI

By Morris Moore

The Barnard Free Skin and Cancer Hospital, the Barnes Hospital, and the Department of Dermatology, Washington University School of Medicine St Louis, Missouri

The necessity for classifying pathogenic fungi is no less urgent or important than in the cases of plant life in general and animal life The confusion existing in man made attempts at the orderly arrangement in a phyletic scheme of pathogenic fungi, however, has made it essential, more than ever, that such a classification be clearly defined and established

To evaluate properly the classification of pathogenic fungi, it would be in order to set forth the criteria by which the taxonomist is necessarily governed in arriving at a justifiable classification Of prime importance is morphology The recognition of the various forms and cell structures which serve to make up the organism and consequently relegate it to an established genus and species is a chief factor Much depends on the interpretation of cell forms, especially in the absence of the perfect structure, the result of the sexual act The size and shape of a specific cell structure, such as the macroconidium or fuseau, is used by some as a means of differentiating the genera *Microsporum*, *Trichophyton*, and *Epidermophyton* (FIGURE 1)¹ Where the perfect form is present, and this is a rarity with pathogenic fungi, the classification of the fungus is relatively simple Since most of the pathogenic fungi are members of the Fungi Imperfecti, classification depends on morphologic characteristics, including methods of spore formation, types of cell structures, location of spores, and special organs As important as morphology is, however, variations may result from alteration of environmental or physical phenomena and consequently must be viewed and interpreted with caution

Gross cultural characteristics, like microscopic morphology, play an important role in diagnostic procedure Size shape, color, texture, and surface markings are of value in the macroscopic determination of fungi As in the case of microscopic morphology, gross cultural characteristics may also be altered perceptibly by external factors which should be considered by the investigator

In a study of pathogenic fungi, with classification in view, efforts are directed towards a determination, if possible, of the perfect stage, the sexual act and the resulting structure To this end, the fungus is grown on a medium which might prove to be suitable for such a determination Altered physiologic response of the fungi due to environmental factors plays important roles in such a procedure

What are some of these factors? To name a few, we have various nutritional substances, both organic and inorganic, temperature, oxygen requirements hydrogen ion concentration, moisture, liquid or solid mediums, various physical factors such as visible light, infra red rays Grenz rays, Roentgen rays (X rays), radium emanations, and perhaps radioactive isotopes To these must be added genetic factors such as may be observed in cytologic studies and in single spore cultures and which may also be altered

by environment The additional use of chemical and biologic procedures are important diagnostic criteria



FIGURE Ma o on d a o fuseaux of dermatophytes 1 *M o po um can* 2 *Trich phy n mentag ph* 3 *Epide mophy on fl o um* 4 *A ho son s hoenle n*

The nutritional requirements of fungi vary considerably according to the type of fungus tissue involved degree of invasiveness obligatory parasitism facultative parasitism and whether the organism exists as a saprophyte within the host or on vegetation outside the host and may consequently be air borne A med um satisfying the needs of one fungus may have little

or no value for another. Much has been written concerning media and their constituents. The inorganic or mineral constituents of media and their utilization by various fungi have been well investigated chiefly for plant pathogens and nonpathogens. Very little has been done in this regard for human pathogenic fungi, perhaps with the exception of calcium, which was considered to be essential for *Trichophyton interdigitale*.[2] In general, the requirements of fungi for inorganic elements is comparable to those of higher plants. Variations in mineral constituents may alter perceptibly the fungus growth. Organic nutrient requirements, like the inorganic elements, play an important part in the growth essentials of pathogenic fungi. The value of the organic material depends upon its assimilability by the fungi. This in turn depends upon the ability of the fungi to produce or secrete an enzyme which will break down the compound so that the breakdown product can be utilized. To complete the cyclic chain, the production of the required enzymes probably depends upon the pathogenic living conditions of the fungus. The alteration of the organic constituents and the inability of the fungus to utilize them will effect the growth characteristics of the fungus. In addition, there are a number of so-called growth factors, vitamins or specific elements, which play an important part

and the perfect stage.

No attempt will be made either to describe or to list the numerous preparations in use, past or present. It would be well, however, to mention some of the media in common use. The closest approach to a universal medium is the modified Sabouraud's agar. As originally prepared by Sabouraud, the *milieu d'épreuve* consisted of raw Chanut maltose, granulated Chassaing peptone, agar, and water. Dextrose could be substituted for the maltose. Sabouraud's conservation agar omitted the carbohydrate,

sively by Sabouraud to produce the giant colonies which served as a basis for identification of the dermatophytes. Unfortunately, this medium contained ingredients which were chemically impure, unstable, and often of

ize luxuriance of

The chief criti-

fact that it was

countries to produce a medium which would duplicate the cultures on Sabouraud s agar resulted in a large number of preparations which failed to attain the objective The use of honey, as advocated by Sabouraud likewise introduced a variable form of nutriment with its source of error In this country the Pennsylvania medium has been advocated but the use of crude glucose although giving good results again introduces a possible source of error

In the United States there is in widespread use a medium also called Sabouraud s agar but which actually may be considered as a completely new substratum It is made up of peptone carbohydrate (glucose or mal

Digestive Ferments Company under the name of Difco Some laboratories prefer the Pfanstiehl sugars which are of a better grade The Difco or Bacto peptone may be criticized on the ground that it is too near neutral and does not aid in developing the giant colonies illustrated in Sabouraud s Les Teignes It has also been condemned by Linder[3] on the ground that Peptone or peptone and sugar in agar, as used in the past are not only unbalanced and do not furnish all the elements necessary for the normal growth of the fungi but when these substances are supplied they are in too great concentration Weidman[4] may perhaps justifiably find fault

usual concentration it is a medium which serves for both rapid isolation and identification of the fungi commonly encountered both in the clinic and in referred patients To be sure this medium does not satisfy the needs of all the fungi Furthermore it does not bring out all the characteristics desirable and still requires the use of additional media for further study of some fungi As an all round medium however it serves the purpose well I am fully aware too of some of its other limitations but then to be able to do medical mycological examinations one needs to have some knowledge of the clinical features of the lesion to be cultured and to use such additional special media as are required To deal with a pure standardized medium means that one must set up special criteria for the recognition of the fungi on this medium The universal adoption of such a medium whether it be this medium or any other substratum satisfying the general needs of pathogenic fungi would eliminate much of the dissension among the mycologists who base their identification and classification on a single medium

Without going into further and greater detail on the very important subject of media I would like to point out again that the ingredients of a medium determine in part the type of growth that will develop Substrata rich in nutritive value are as a rule to be avoided since for the most part they develop vegetative mycelia without sexual structures Poor media on the other hand are in use to try to induce free sporulation and

the formation of the sexual aspects of the fungus In most common use are the gypsum blocks, various vegetable plugs, and Gorodkova's agar On the basis of studies made of *Ctenomyces serratus* growing naturally on feathers, it has been suggested that the dermatophytes are related to, or probably members of, the *Gymnoascaceae* of the *Plectascales* Because of the similarity of certain structures of this organism, such as spirals and aleurospores, to those seen in the dermatophytes, it has been proposed that

be attributed with certainty either to the peculiar substratum or to the age of the cultures (four months)

There is evidence that temperature is of definite value in both growth and development of fungi and in the production of sexual characteristics In general, it has been shown that somewhat lower temperatures are required for the formation of sexual organs than are necessary for vegetative mycelia Most of the work has been done on nonhuman pathogens, but, for all intents and purposes, it may be assumed that the same applies to human pathogenic fungi The growth at incubator temperature simulates the vegetative phase of the parasitic state in the human host and, as such, has little value for botanical classification Consequently, growth at room temperature, approximately 22° C, is much more to be desired, although that may not be the optimum temperature for the specific organism

as is the case with tight plugs or plugs dipped in paraffin or screw caps for

israeli (*A bovis*) will grow only in a microaerophilic state This, of course, brings up the question as to whether one should classify a fungus purely on

genera purely on the basis of oxygen requirements The value of partial anaerobiosis has been well demonstrated in the case of *Coccidioides immitis*, where the development of endosporulation *in vitro* takes place only under partial anaerobic conditions [6]

Hydrogen ion concentration (pH)

portant bearing Talice[7] studied a number of pathogenic fungi grown on

three different media with varying hydrogen ion concentrations, and indicated the pH at which minimum, optimum, and maximum growth took place He noted frequently gross variations in the form and color of the colonies especially in the highly tinted species and, oftentimes, variations [illegible]

[illegible]

cation Most organisms especially yeasts and yeastlike forms require moisture both for growth and for the germination of spores Liquid media are used for both the study of spore germination and the production of certain chemicals and vitamins which may be elaborated in a medium as a result of the growth processes of special fungi Since, among the Fungi Imperfecti classification depends to some extent on the sporulation of the fungus moisture is of great importance At the present time we employ both liquid and solid media The solid substrata used today, however, are chiefly [illegible]
media [illegible]
except [illegible]
slower unless stimulated by temperature changes Such media often produce an abundance of vegetative mycelia but are of less value for the formation of reproductive or sexual organs Occasionally, too, various spores require alternate wetting and drying for maximum germination Although wet media are the rule in mycology, very often old dried-out cultures develop specialized structures such as sclerotia, which may help considerably in arriving at a classification of the fungus

Various physical factors have been employed in mycology, but chiefly to determine their lethal effect on fungi These include visible light infra red rays ultraviolet rays Grenz or infraroentgen rays, Roentgen rays (X rays) and radium emanations Visible light has been shown to have a definite morphogenic influence on certain fungi as well as to stimulate the development of reproductive structures Pathogenic fungi, in general are very little affected by visible light Most of the work done on fungi has been with the rays of shorter wave lengths Smith,[8] in 1936 induced variants in fungi experimentally by using suitable exposures of ultraviolet radiations Hollaender and Emmons[9] (also Emmons and Hollaender[10]), working with the effect of measured amounts of monochromatic ultraviolet radiation on spores of the dermatophyte *Trichophyton mentagrophytes*, found that wave lengths of 2280 to 2950 Ångstroms affected these spores The lethal effect and the secondary mutant formation occurred with wave lengths of 2537 and 3650 Ångstroms with a maximum of 100×10^{-4} ergs per spore (With measured amounts and increased time of exposure, the amount of mutations decreased) The effect of the sublethal radiation manifested itself by the production of small, slow growing colonies The spores that survived the lethal dose produced colonies which resulted in the mutants These mutants differed from normal controls in their size, form rate of growth, spore production and color Unfortunately genetic studies could not be

ıade because of the lack of the perfect stage, the sexual organs Spores of ld cultures, however, were able to produce similar mutants

Muskatblit and Ouspensky,[11] using Grenz rays on hairs affected with *Microsporum audouini*, *M canis*, *Trichophyton crateriforme*, *T violaceum*, nd *Achorion (Trichophyton) schoenleinii*, found doses up to 50,000 roentgens neffective on the gross or microscopic morphology of these fungi Fungi xposed to Roentgen or X rays showed changes Some of the colonies deeloped color changes, slow rate of growth, and small amount of mycelial levelopment with colony sectoring Many of the fungi were considered to e saltants, since the effects of the radiation wore off in subsequent subultures Others, however, persisted as mutants Sartory, Sartory, and Meyer[12] exposed *Aspergillus fumigatus* to 3–7 2 millicuries of radium emana ions and were able to induce morphologic changes in the organism Largeelled oidia, thick walled spores, and large pseudosporangia were produced n media that were practically salt free When cultures were exposed to ıigher doses, hard, fusiform sclerotia containing perithecia were found in he subsurface mycelium It is evident that physical factors may induce norphologic and, perhaps, genetic changes in fungi in sufficient amount to ıave an effect on the classification of these organisms No doubt additional

eration that most pathogenic fungi belong to the Fungi Imperfecti, and genetic studies cannot be made because of the lack of suitable material, such as the ascospores of *Neurospora* which have been so fruitful for so many

cation Monospore cultures such as were made by Emmons for *Microsporum gypseum* (*M fulvum*) are a step in this direction Certainly the

has been so, perhaps, because of the technical procedure that is involved in making such a study In the Fungi Imperfecti, cytological investigations would amply repay the worker with the vast amount of information that

possible that light would be shed on the reproductive organs or processes by investigating the various cellular forms of the fungi under varying con-

three different media with varying hydrogen ion concentrations and indicated the pH at which minimum, optimum, and maximum growth took place. He noted frequently gross variations in the form and color of the colonies, especially in the highly tinted species and, oftentimes, variations in the microscopic characters of the fungi. Microscopically, he noted a predominance of one form of vegetation according to the pH value.

Moisture is usually a requisite in mycological investigation and classification. Most organisms, especially yeasts and yeastlike forms require moisture both for growth and for the germination of spores. Liquid media are used for both the study of spore germination and the production of certain chemicals and vitamins which may be elaborated in a medium as a result of the growth processes of special fungi. Since, among the Fungi Imperfecti classification depends to some extent on the sporulation of the fungus, moisture is of great importance. At the present time, we employ both liquid and solid media. The solid substrata used today, however, are chiefly of the nature of colloidal gels, with the exception of specialized solid media. These colloidal gels require moisture and as such serve the purpose, except that germination of spores or free spore formation is usually much slower unless stimulated by temperature changes. Such media often produce an abundance of vegetative mycelia but are of less value for the formation of reproductive or sexual organs. Occasionally, too, various spores require alternate wetting and drying for maximum germination. Although wet media are the rule in mycology, very often old, dried-out cultures develop specialized structures, such as sclerotia, which may help considerably in arriving at a classification of the fungus.

Various physical factors have been employed in mycology, but chiefly to determine their lethal effect on fungi. These include visible light, infra red rays, ultraviolet rays, Grenz or infraroentgen rays, Roentgen rays (X rays) and radium emanations. Visible light has been shown to have a definite morphogenic influence on certain fungi, as well as to stimulate the development of reproductive structures. Pathogenic fungi, in general are very little affected by visible light. Most of the work done on fungi has been with the rays of shorter wave lengths. Smith,[8] in 1936, induced variants in fungi experimentally by using suitable exposures of ultraviolet radiations. Hollaender and Emmons[9] (also Emmons and Hollaender[10]), working with the effect of measured amounts of monochromatic ultraviolet

of 2537 and 3650 Ångstroms with a maximum of 100 × 10^{-4} ergs per spore. (With measured amounts and increased time of exposure, the amount of mutations decreased.) The effect of the sublethal radiation manifested itself by the production of small slow growing colonies. The spores that survived the lethal dose produced colonies which resulted in the mutants. These mutants differed from normal controls in their size, form, rate of growth, spore production and color. Unfortunately, genetic studies could not be

made because of the lack of the perfect stage, the sexual organs Spores of old cultures, however, were able to produce similar mutants

Muskatblit and Ouspensky,[11] using Grenz rays on hairs affected with *Microsporum audouini, M canis, Trichophyton crateriforme, T violaceum,* and *Achorion (Trichophyton) schoenleinii*, found doses up to 50,000 roentgens ineffective on the gross or microscopic morphology of these fungi Fungi exposed to Roentgen or X rays showed changes Some of the colonies developed color changes, slow rate of growth, and small amount of mycelial development with colony sectoring Many of the fungi were considered to be saltants, since the effects of the radiation wore off in subsequent subcultures Others, however, persisted as mutants Sartory, Sartory, and Meyer[12] exposed *Aspergillus fumigatus* to 3-7 2 millicuries of radium emanations and were able to induce morphologic changes in the organism Large celled oidia, thick-walled spores, and large pseudosporangia were produced on media that were practically salt free When cultures were exposed to higher doses, hard, fusiform sclerotia containing perithecia were found in the subsurface mycelium It is evident that physical factors may induce morphologic and, perhaps, genetic changes in fungi in sufficient amount to have an effect on the classification of these organisms No doubt additional work will be done with these agents, to which may be added the recently developed radioactive isotopes, which, at present, are an unknown quantity in many fields of biologic endeavor

One branch of biology that has been sadly neglected in medical mycologic classification and in its general application to pathogenic fungi is cytology and its genetic implications This may be attributed chiefly to the consideration that most pathogenic fungi belong to the Fungi Imperfecti, and genetic studies cannot be made because of the lack of suitable material, such as the ascospores of *Neurospora* which have been so fruitful for so many [illegible]

cation Monospore cultures such as were made by Emmons for *Microsporum gypseum* (*M fulvum*) are a step in this direction Certainly the

fungi This phase has been passed over too lightly by mycologists This has been so perhaps, because of the technical procedure that is involved in making such a study In the Fungi Imperfecti, cytological investigations would amply repay the worker with the vast amount of information that could be obtained In the absence of essential criteria which make classi-

by investigating the various cellular forms of the fungi under varying con-

ditions of growth Cytological investigations should be highly recommended to the young enthusiasts of mycology

In addition, there are other procedures which are essential if pathogenic fungi are to be classified properly These include various biochemical studies such as fermentation, especially useful in the case of yeastlike organisms, assimilation of carbon and of nitrogen, gelatin liquefaction, hydrolysis of starch, indol production, and others, some of which may or may not have practical value In addition, biological and serobiological reactions have also been applied in mycological classification, and these include agglutination determinations, immunological reactions, and others

What has been presented so far may be summarized briefly as representing the tools of the mycologist for the determination and classification of pathogenic fungi How wisely these tools are used and how well the results are interpreted will determine the accuracy of the diagnosis and classification

To keep the botanist from straying too far from a logical and sane system of classification, a binomial nomenclature has become established and is being maintained by the taxonomists, who are guided by a set of International Rules of Botanical Nomenclature These have been reprinted by Dodge[13] with examples pertaining to pathogenic fungi Many pleas have already been made for strict adherence to and complete adoption of these rules Needless to say, those of us who are familiar with these rules seek to do all we can to respect them Inadvertently, however, most of us have at one time or another been guilty of either neglect, forgetfulness, or unconcern Much of the confusion existing in our present system of classification has arisen from the simple fact that these rules have been disregarded and personal interpretations have been substituted These rules are as perti

for newly described fungi makes the new name invalid While this is important from the standpoint of taxonomists, there are other rules of great significance which have been disregarded

The avoidance and violation of the principle of priority has been responsible for a share of the chaos in the classification of pathogenic fungi This rule refers to the maintaining of the oldest and first binomial given to a group or species Unfortunately, this infraction could be excused on the ground that it was unintentional or that the early descriptions were brief, incomplete, and poorly illustrated, or that they appeared in a journal which was not readily accessible Since, in many instances, the original publications are not seen the investigators depend upon the quotations present in the papers of others Interpretations based on such descriptions varied according to the group studying them, and, as a consequence, there have arisen groups or schools who follow the interpretations or concepts of certain leaders As a result of such conceptions or misconceptions, it is not unusual to find that the name applied to the organism by the original worker may be used by present workers to refer to an entirely different fungus I be-

lieve the term *gypseum* is one which has been responsible for this type of confusion An outstanding example of the misuse of terms is that concerned with the agent or agents of moniliasis The term *Monilia*, which has been applied to the organism of moniliasis and which is falling into disrepute, was adopted as the cause of this disease (FIGURE 2) The present day description of this genus and its usage as related to pathogenic fungi is totally different from the original concept The creation of the genus *Syringospora* by Quinquaud in 1868, based on the *Oidium albicans* of Robin is undoubtedly the first valid and legal name for the organism of moniliasis and as such should replace the various genera being used for the organism in question As a result of the misinterpretation of the older legalized terminology, there developed a type of sentimentality in classification with

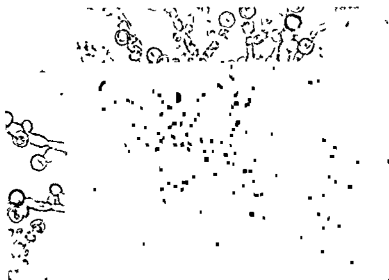


FIGURE 2 *Syringospora albicans* grown on corn meal agar Note large characteristic chlamydospores

each group of workers adhering to the ideas and ideals of the exponents of these groups Consequently, we find that many adhere to the genus *Candida* of Berkhout published in 1923 which in its present-day usage refers

Syringospora, but these have been referred to the genera *Mycotorula Candida* or *Monilia*[14] Many of the species have been either reduced to synonomy or eliminated but there are still other species and genera which need classification through monographic study

Another outcome of the incomplete descriptions or poor illustrations of the earlier workers has been the development of two widely separated

groups, the "lumpers" and the "splitters" The lumpers tend to decrease the number of genera and species to a few, whereas the splitters favor the creation of new genera and species based on the interpretation of new or different characters Simplification of classification is, of course, highly commendable and it makes the remembering of names fairly easy, especially for the none too botanically minded medical men On the other hand we must avoid oversimplification, for, in the end, this will defeat the purpose of taxonomy The splitters may be criticized for the creation of too many genera and species, although some may be justifiable A compromise group is the ideal, and this should be the goal of the taxonomists

A recent example of this is illustrated by the organisms causing chromomycosis or chromoblastomycosis On the basis of the various characters produced in culture, the fungi were provisionally separated into several genera, *Phialoconidiophora*, *Phialophora*, *Hormodendroides*, *Botrytoides* and *Hormodendrum*, and placed in the order *Phialophorae* at a time when there was a state of confusion existing in the classification of these pathogens (FIGURE 3)[15] Because of the various morphological characteristics in culture, the fungi had been variously classified as *Acrotheca*, *Gomphinaria*, *Trichosporium*, *Hormodendrum*, and then *Fonsecaea* In contrast, one of the recent publications would place all the organisms with their assorted characteristics in the one genus, *Phialophora*, on the basis of finding the cupuliform spore bearers in all the strains[16] I believe that this group of fungi needs further study for final disposition

In some instances the choice of a name has led to the discarding of the

we are not in favor of the name given these microorganisms, let us strive to attain at least some semblance of order by retaining the legitimate name given by the investigator In this regard, the rules state clearly that "a name or epithet must not be rejected, changed or modified merely because *it is badly chosen, or disagreeable or because another is preferable or better* known" There have been numerous instances of this violation in the classification of pathogenic fungi

The rule stating that only one name may be given to an organism regardless of how many pleomorphic forms or life cycles it may have does much to alleviate the situation concerning the dermatophytes and other fungi with a variable life cycle It would seem, therefore, that at best the classification of most pathogenic fungi is probably tentative, subject to the eventual finc

matter how

of the binor

stage is associated An example of this may be cited in the case of *Monosporium apiospermum*, the perfect stage of which is reputedly *Allescheria boydii*[17]

The present system of classification is based on the work of Saccardo, as shown in his "Sylloge Fungorum" An attempt was made to bring to-



F G RE 1 Organisms of Chromomycosis. 1 *Phialophora verrucosa* 2 *Hormodendrum p* 3 *Hormodendrum de ped o* 4 *Phialoconidiophora guggenheimia* 5 *B y oides monophora*

then an attempt is made to follow a natural system of evolution with the most complex form of life being the latest and the simplest form being the oldest The division of the fungi into classes orders families genera and species is essentially an indication of relationship based on the evolutionary

processes Although the tendency is to consider it as following natural lines much of it may be considered to be artificial until more concrete evi dence is presented Most pathogenic fungi do not present a significant amount of evidence on which to base a phyletic sequence except as relates to individual groups Thus before we can assume a definite system of classification such as exists with other fungi we must diligently apply our selves to the investigation of these fungi Since the present classification of fungi is man made we should not expect it to be perfect, and there are those who may accordingly disagree with the form and substance of such a classification Any attempt to alter the system for the better should therefore be given careful and encouraging consideration

Pathogenic fungi for the most part have been isolated studied and clas sified by medical men who patterned their classification chiefly along clinical lines The particular fungus was related to the clinical features of the d s ease both macro- and microscopically The monumental work of Sabou raud and of the earlier workers who helped lay the cornerstone for the classification of the dermatophytes and of medical mycology in general followed very much along natural lines (FIGURE 4) The value of their work undoubtedly is great so great that from a clinical standpoint and from the point of view of the dermatologist it has not been surpassed and is still in use This, of course brings up the question of whether the classification of pathogenic fungi should be made to fit the needs of the medical man or of the pure mycologist It is desirable to have a classification which would facilitate diagnosis and from an economic and practical viewpoint help both the medical man and the patient The difficulty with such a class fication however lies in the fact that the diversity of lesions that a fungus

mediums developed a compromise classification On the other hand Em

at some points In spite of the confusion that exists as a result of such classification I feel that such difficulties will be ironed out in due course

sed on the char
the logical and
their pathogenic

state however fungi present certain criteria which help to place them in certain groups To this end we should consider the arbitrary clinical clas sification of mycoses and their causative agents based on anatomic distri bution and also on their ability to invade tissue Briefly mycoses may be considered clinically as belonging to one of several groups (1) superficial mycoses affecting only the *stratum corneum* of the skin or the hair never becoming invasive or systemic (2) dermatomycoses involving and invad ing the superficial and deep layers of the skin and also the hair and nails

TABLEAU SYNTHÉTIQUE DES DERMATOPHYTES

- **I. MICROSPORUMS**
  - Microsporums purs de type humain
    - *Microsporum Audouini*
    - M. umbonatum
    - M. tardum
    - M. velveticum
  - Néo-microsporums ou d'origine animale conservant longtemps leur type parasitaire jeune
    - Microsporum *lanosum*
    - M. felineum
    - M. equinum
    - M. fulvum
    - M. villosum
    - M. pubescens
    - M. tomentosum
- **II. TRICHOPHYTONS**
  - Endothrix
    - Endothrix purs
      - Espèces types fréquentes
        - Trichophyton *crateriforme*
        - Tr. *acuminatum*
        - Tr. *violaceum*
      - Espèces rares ou étrangères
        - Tr. effractum
        - Tr. fumatum
        - Tr. umbilicatum
        - Tr. regulare
        - Tr. *sulfureum*
        - Tr. polygonum
        - Tr. exsiccatum
        - Tr. circonvolutum
        - Tr. pilosum
        - Tr. glabrum
    - Néo-endothrix
      - Conservant le type parasitaire de la période jeune
        - Trichophyton *cerebriforme*
        - Tr. plicatile
  - Ectothrix
    - Microïdes
      - Type *gypseum*
        - Trichophyton *asteroïdes*
        - Tr. radiolatum
        - Tr. lacticolor
        - Tr. granulosum
        - Tr. farinulentum
        - Tr. persicolor
      - Type *niveum*
        - Trichophyton *radians*
        - Tr. denticulatum
    - Mégaspores
      - A culture *veloutée*
        - Tr. *rosaceum*
        - Tr. vinosum
        - Tr. *equinum*
        - Trichophyton caninum
      - A culture *faviforme*
        - Trichophyton *ochraceum*
        - Tr. *album*
        - Tr. discoïdes.
- **III. ACHORIONS**
  - Achorion du favus humain
    - *Achorion Schonleinii*
  - Achorions animaux
    - A. Quinckeanum
    - A. gallinae
    - A. gypseum
    - Oospora canina.

(there is no evidence of systemic involvement), and (3) mycoses usually primarily involving the skin or mucous membranes or both, systemic invasion being either primary or secondary and systemic infections, chiefly primary with occasional or rare secondary cutaneous involvement

Fungi producing disease in the first group are fortunately, few in number and present well-established forms which make them easily recognizable Although there is some disagreement as to the naming of these fungi it is only a matter of time and careful study of these organisms before definite taxonomic differentiation will be made In the second group the dermatophytes, careful study of the fungi in the parasitic state will reveal sufficient differences in morphology to allow for allocation at least to genera In many instances however, because of age and various environmental factors the physiology of the fungi has become so altered that morphologic

perhaps the greatest amount of attention, there are still several points that need elucidation Here a good knowledge of the clinical aspects of the disease plus a background of general mycology, as well as medical mycology, is of tremendous importance in classification

Organisms producing disease of the internal organs or a combination of cutaneous, mucous membrane and systemic lesions are, in general, recognized with relative ease There are, however, several criteria which play an important part and may easily confuse the not too well trained Fungi seen in tissue blood pus or cerebrospinal fluid at body temperature develop morphologic structures which remain fairly constant in the parasitized host The organisms in this state present the parasitic phase of the life cycle As such they may be classified as to groups and type of disease but give no structures which can be classified botanically Whole blood serum and tissue extracts are utilized in artificial media and maintained at body temperature in the incubator to continue this phase of the fungus life cycle Although this serves the purpose described the specific classification of the organism cannot be accomplished by this means A frequent error in the classification of fungi in the parasitic state is in referring to all budding organisms as blastomycetes" The term blastomycete brings to mind the misnamed organism of blastomycosis, *Blastomyces dermatitidis* It is readily understandable therefore that such means of classification are not only misleading but also confusing Several fungi show budding forms in

URE 5) Obviously such confusion can be dispersed by growing the organism on a suitable medium which will bring out specific characteristics More significant however, would be to discard the term '*Blastomyces* which rapidly is assuming the role of a *nomen ambiguum*

Much progress has been made in the classification of pathogenic fungi Much more will have to be done to develop a readily understandable work

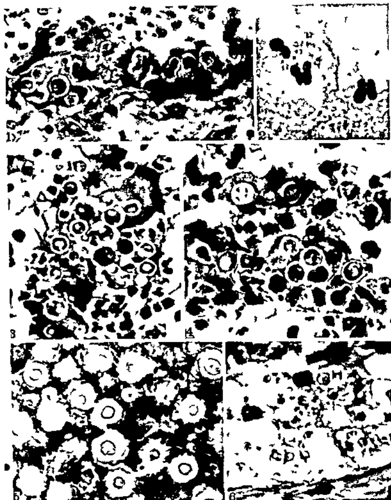


FIGURE 5 Budding yeastlike pathogenic fungi in tissue 1 *Zymonema dermatitidis* in the adrenal 2 *Syringospora albicans* in nail scraping 3 *Paracoccidioides brasiliensis* in lymph node 4 *Histoplasma capsulatum* in the prostate 5 *Cryptococcus neoformans* in the brain 6 *Sporotrichum schenckii* experimental inoculation in the mouse testis

help considerably to facilitate clinical diagnosis in a language readily understandable by the clinician To help alleviate the confusion existing in the botanical classification the adherence to a set of guiding rules such as pro

posed by the Committee on International Rules of Botanical Nomenclature is essential for uniformity of classification throughout the civilized world I feel that an international subcommittee devoting itself to pathogenic fungi exclusively and to which new organisms with their new designations could be submitted for final disposition would eliminate future confusion At the same time I feel that some provision should be made for a clinical classification which would benefit the medical man and yet not hamper the botanical classification of pathogenic fungi This perhaps is a big order but I firmly believe that the one may complement the other and yet each be valuable individually

From the foregoing discussion one may reasonably conclude that the status of the classification of pathogenic fungi is as yet unstable and is replete with confusion and chaos To be sure, we have not attained the perfection in classification that is to be desired, but the future is not dark As long as we have workers interested in the classification of pathogenic fungi and trained mycologists who are willing to adopt the dynamic methods of classification, then confusion will be dispelled and order will reign Medical mycology is still a young science, and where there is youth there is hope

Bibliography

1 Emmons C W 1934 Arch Dermat & Syph **30** 337
2 Mosher W A D H Saunders L K Kingery & R J Williams 1936 Plant Physiol **11** 795
3 Linder D H 1941 Mycologia **33** 453
4 Weidman F D & T M McMillan 1921 Arch Dermat & Syph **4** 451
5 Emmons C W 1932 Arch Dermat & Syph **25** 987
6 MacNeal W J & R M Taylor 1914 J Med Res **30** 261
Moore M 1932 Ann Missouri Bot Gard **19** 397
Ciferri R & P Redaelli 1934 Boll Sez Italiana Soc Internaz Microbiol **6** 141
Lack A R 1938 Proc Soc Exp Biol & Med **38** 907
7 Talice R V 1930 Ann Parasitol hum et comp **8** 183
8 Smith E C 1936 Biological Effects of Radiation 889–918 (Ed B M Duggar) McGraw Hill New York
9 Hollaender A & C W Emmons 1939 J Cell & Comp Physiol **13** 391
10 Emmons C W & A Hollaender 1939 Am J Bot **26** 467
11 Muskatblit E & B Olspensky 1933 Arch Dermat & Syph **27** 953
12 Sartory, A R Sartory & J Meyer 1926 C R Acad Sci **183** 77 *idem* 1926 C R Acad Sci **183** 1360
13 Dodge C W 1935 Medical Mycology Fungous Diseases of Man and Other Mammals 75–96 C V Mosby Company St Louis
14 Langeron, M & R V Talice 1932 Ann Parasitol hum et comp **10** 1
Martin D S C P Jones K F Yao & L E Lee Jr 1937 J Bact **34** 99
Conant N F 1940 Mycopathologia **2** 258
Martin D S & C P Jones 1940 J Bact **39** 609
Almeida F de C de Lacaz & O de Barros 1941 Rev Inst Adolfo Lutz **1** 395

FUTURE DEVELOPMENTS IN MYCOLOGICAL INVESTIGATIVE METHODS

By Norman F Conant

Department of Bacteriology Duke University School of Medicine Durham, North Carolina

Although a fungus was the first recognized infectious agent of man and animal, relatively little is known about fungus diseases in comparison with the accumulated knowledge concerning the epidemiology, immunology, immunochemistry, and specific therapy of bacterial and virus infections The lack of such information about fungus infections has been the result of methods of study which stressed the clinical aspects of the diseases and the taxonomy of the fungi almost to the exclusion of fundamental studies of the infectious agents and the disease processes which they caused At the present time, however, there are few disagreements concerning the pathogenicity and the taxonomy of those fungi which produce superficial or systemic mycoses The dermatophytes are no longer represented by a vast, complicated, and confused group of organisms Rather, they have been reduced to a small number of easily recognized and identified fungi The systemic fungi, likewise, have evolved through a period of recognition, classification, and confusion, and now are also known to be represented by a small number of easily recognized and identified fungi It is hoped, therefore, that investigative methods may now be directed more fully towards gaining a better understanding of the physiological chemical, immunological, and epidemiological aspects of the human pathogenic fungi and the diseases they produce

A trend toward the use of such mycological investigative methods has already been forecast by recent studies which have made use of these tools For example, growth requirements of the fungi are no longer studied by changing brands of peptone and types of sugar in the media Robbins and Ma (1942, 1944, 1945) have investigated the nutritional requirements of *Trichophyton discoides*, *Rhodotorula sp*, and *Trichophyton mentagrophytes* by their behavior on a synthetic basal agar medium or synthetic basal solution

stimulate conidial production and colony formation A further study of the exact requirements for a better growth of *M audouini* is to be reported later Since microscopic morphology and gross cultural characteristics are used to identify the pathogenic fungi, the stabilization of such characters by growth on a synthetic medium to which can be added known substances would allow duplication and a more stable taxonomy The value of a method of study that would lead to such a stabilization is of course, readily apparent

Physiological investigations of the human pathogenic fungi also have made use of methods that provide basic quantitative information concerning the activity of the organisms Bernheim (1942), Nickerson (1946), and

Nickerson and Chadwick (1946) have indicated the value of respiration studies (oxygen consumption) as a method of measuring the *quantitative* effect of toxic substances on living, pathogenic fungi. Investigations of this type might also indicate the enzyme system or systems involved in the oxidation of various substances when drugs are added to the preparations and oxidation is hindered. This type of information, obtained by such studies, could lead to definite points of attack against the pathogenic fungi, since *it not only gives quantitative data about the toxicity of drugs but also* indicates their mode of action.

Chemical investigations of the human pathogenic fungi are needed to yield valuable information concerning the metabolic products which they produce and to which allergy or immunity is established in the infected human or animal. Trichophytin, coccidioidin, blastomycin, and, lately, histoplasmin, are sterile filtrates of a liquid medium in which the respective fungi have been grown for long periods of time. Such materials, when injected intradermally, give delayed tuberculinlike reactions which denote present or past infection by the homologous fungi.

Purified products of some of these materials have been obtained and comparable studies with the "crude" filtrates have been made. "Crude" trichophytin and its purified products have been used in studies relating to infection, immunity, and sensitization caused by the dermatophytes. Reviews and personal investigations contained in reports by Sulzberger (1932), Jadassohn, Schaaf, and Wohler (1937), DeLamater and Benham (1938), and DeLamater (1941) have shown that infection by this group of fungi causes sensitization that can be demonstrated by skin tests, and that these fungi contain common or group antigens. Infection by one member of the group, therefore, may cause sensitization that can be demonstrated by a skin test, not only to the homologous extract but also to an extract of other *dermatophytes. Extracts of fungi not belonging to this group* were found not to cross react.

There have been recent reports, however, which indicate that some of the dermatophytes produce antigens or reagins which sensitize the skin to products produced by markedly different fungi. For example, penicillin allergy in patients who have never received penicillin is thought to be established by previous infection of the skin by species of dermatophytes. Peck and Hewitt (1945) showed that some dermatophytes produce a penicillinlike substance, and Cormia and Lewis (1946) showed a correlation between penicillin sensitivity and sensitivity to superficial fungus infections. Peck and Siegal (1947) attribute penicillin sensitivity to an infection of the *skin by dermatophytes which produce the penicillinlike substance* which, in turn, creates the sensitivity that is elicited when penicillin is used therapeutically. These later studies show, therefore, that widely different fungi do contain common antigenic substances that may prepare patients for later allergic manifestations. These facts have great clinical significance, and more should be known about the types of products produced by the dermatophytes, since these fungi cause widespread infection in the population.

Although the sensitizing antigens contained in trichophytin have been studied extensively, not much is known about the same types of materials that may be present in extracts of fungi which cause systemic infections *Coccidioides immitis* is known to produce a substance or substances in broth cultures which cause a specific reaction in patients and experimentally infected animals This material, coccidioidin, may also be used as the antigen in complement fixation tests and in precipitin reactions Hirsch and Benson (1927) showed filtrates of cultures of *C immitis* to give specific skin

coccidioidin by alcohol precipitation More recently, Hassid, Baker, and McCready (1943) isolated a polysaccharide from coccidioidin, which gave positive skin tests to 0 00001 mg injected intradermally and gave a precipitate in serum and pleural fluid in a dilution of 1.640,000 These few reports indicate the nature of the specific substance in culture filtrates of *C immitis* The "crude" filtrate, coccidioidin, has been used, however, as a specific skin testing material in epidemiological studies to determine the rate of infection in the population and to determine the endemic foci of coccidioidomycosis in this country The clinical significance of an estab

has been reported by Martin and Smith (1939) They have shown that patients sensitive to a skin test dose of a standardized heat killed *Blastomyces* vaccine could not be given iodides safely without previous desensitization In such patients, infection spread rapidly when iodides were given in the presence of sensitivity to the organism Peck, Martin and Hauser (1940) isolated polysaccharides from the yeast phase of *B dermatitidis* and found that this type of material gave positive skin tests and also could be used for desensitization The allergic state of the patient in this disease, therefore, must be established before treatment can be instituted Not only should the allergic state of a patient infected with *B dermatitidis* be established before rational treatment can be given, but the immunologic status of the patient should also be known Martin and Jones (1941) were

was thought to have excess antigen produced by the fungus, which masked the skin test and inhibited antibody formation By injecting immune rabbit serum the patient s immunologic status was reversed and response to treatment was immediate A similar type of immunologic finding has also been reported by Hiatt and Martin (1946) in a patient with pulmonary moniliasis Rapid clearing of the lungs followed treatment with anti-albicans rabbit serum in a patient showing a positive Foshay type of skin

test to immune serum, but in whom there was a negative skin test to vaccine and a negative agglutination test. In this patient, it was thought that excess antigen produced by *Candida albicans* masked the skin test and inhibited antibody formation. In these two instances, therefore, an understanding of the patient's immune response was necessary for successful treatment. Information concerning the significance of sensitivity and immune responses in other types of systemic fungus infections may prove equally as important.

While the allergic and immune status of an individual patient should be studied with all of the available techniques, it is also of great importance to establish the rate of infectic ation for a better understanding (two systemic fungus infections have changed the concept of these diseases. Both were thought to be highly fatal infections until skin test materials were developed that could detect minimal or past infections by eliciting a positive skin test, comparable to and interpreted in the same way as the tuberculin test. Gifford (1936) and Dickson (1937) were the first to demonstrate that a primary benign type of infection was caused by *Coccidioides immitis*. Inhabitants in the known endemic areas of the disease have shown a high incidence of infection, as determined by positive coccidioidin tests. The secondary malignant progressive phase of coccidioidomycosis (coccidioidal granuloma) was found to occur infrequently and to be more prevalent in the dark skinned races.

Until recently, histoplasmosis has been considered a highly fatal infection. Christie and Peterson (1945), Palmer (1945 and 1946), and Furcolow, High, and Allen (1946), however, have shown that in certain parts of the country there is a high correlation between pulmonary calcification in nontuberculin reactors and positive skin tests to histoplasmin. If this skin testing material proves to be specific (Emmons, Olson, and Eldridge, 1945; Howell, 1947), it will have shown that *Histoplasma capsulatum* also causes a primary benign type of infection and that the highly fatal form of the disease represents only a not too frequent secondary malignant type of infection.

It is hoped that the future development of mycological methods will stress these physiological, chemical, immunological, and epidemiological approaches and will yield a better understanding of the human pathogenic fungi. Much has already been accomplished, and the methods of study have been indicated. Such a program will need the help of adequately trained personnel. The personnel necessary for effective work would include a mycologist, a physiologist, an immunochemist, and a pathologist. This group should be in contact with clinical facilities that would provide cases for study and should be in contact with clinicians who would help select the problems to be investigated.

Bibliography

BERNHEIM, F. 194[illegible]. The effect of various substances on the oxygen uptake of *Blastomyces dermatitidis*. J. Bacteriol. 44: 533.

CHRISTIE, A. & J. C. PETERSON. 1945. Pulmonary calcification in negative reactors to tuberculin. Am. J. Pub. Health 35: 1131.

CORMIA F E & G M LEWIS 1946 Experimental aspects of penicillin sensitization with special reference to conjoint sensitization to superficial fungus diseases J Invest Dermat 7 375

DELAMATER, E D 1941 Experimental studies with the dermatophytes III Development and duration of immunity and hypersensitivity in guinea pigs J Invest Dermat 4 143

DELAMATER, E D & R W BENHAM 1938 Experimental studies with the dermatophytes I Primary disease in laboratory animals J Invest Dermat 1 451

DELAMATER E D & R W BENHAM 1938 Experimental studies with the dermatophytes II Immunity and hypersensitivity produced in laboratory animals J Invest Dermat 1 469

DICKSON E C 1937 "Valley fever" of San Joaquin Valley and fungus *Coccidioides* Calif & West Med 47 151

DICKSON, E C 1937 *Coccidioides* infection Part I Arch Int Med 59 1029

EMMONS, C W, B J OLSON & W W ELDRIDGE 1945 Studies of the role of fungi in pulmonary disease I Cross reactions of histoplasmin Pub Health Rep 60 1383

FURCOLOW M L, R H HIGH & M F ALLEN 1946 Some epidemiological aspects of sensitivity to histoplasmin and tuberculin Pub Health Rep 61 1132

GIFFORD M A 1936 San Joaquin fever Ann Rep Kern County Health Dept 22 23

HASSID W Z, E L BAKER & R M MCCREADY 1943 An immunologically active polysaccharide produced by *Coccidioides immitis* Rixford and Gilchrist J Biol Chem 149 303

HAZEN E L 1947 *Microsporum audouini* the effect of yeast extract thiamine pyridoxine and *Bacillus weidmaniensis* on the colony characteristics and macroconidial formation Mycologia 39 200

HIATT J S & D S MARTIN 1946 Recovery from pulmonary moniliasis following serum therapy J A M A 130 205

HIRSCH E F & H BENSON 1927 Specific skin and testis reactions with culture filtrate of *Coccidioides immitis* J Infect Dis 40 629

HIRSCH E F & D D'ANDREA 1927 The specific substance of *Coccidioides immitis* J Infect Dis. 40 634

HOWELL, A JR 1947 Studies of fungus antigens I Quantitative studies of cross reactions between histoplasmin and blastomycin in guinea pigs Pub Health Rep 62 631

JADASSOHN W, F SCHAAF & G WOHLER 1937 Analyses of composite antigens by the Schultz Dale technic, further experimental analyses of trichophytins J Immunol 32 203

MARTIN D S & D T SMITH 1939 Blastomycosis (American blastomycosis Gilchrist's disease) I Review of literature II Report of thirteen new cases Am Rev Tuberc 39 275 488

MARTIN D S & R R JONES 1941 Systemic blastomycosis Report of a case with unusual immunologic findings well two years after onset Surgery 10 939

NICKERSON W J 1946 Inhibition of fungus respiration a metabolic bio-assay method Science 103 484

NICKERSON W J & J B CHADWICK 1946 On respiration of dermatophytes Arch Biochem 10, 81

PALMER C E 1945 Nontuberculous pulmonary calcification and sensitivity to histoplasmin Pub Health Rep 60 513

PALMER C E 1946 Geographic differences in sensitivity to histoplasmin among student nurses Pub Health Rep 61 475

PECK R L, D S MARTIN, & C R HAUSER 1940 Polysaccharides of *Blastomyces dermatitidis* J Immunol 38 449

PECK S M & W L HEWITT 1945 The production of an antibiotic substance similar to penicillin by pathogenic fungi (dermatophytes) Pub Health Rep 60 148

PECK S M & S SIEGAL 1947 Immunologic relationships of the antibiotics and trichophytin Clinical observations and animal experiments J Invest. Dermat 9 165

ROBBINS, W J & R MA 1942 Vitamin deficiencies of *Trichophyton discoides* Bull Torrey Bot Club 69 509

ROBBINS W J & R MA 1944 A *Rhodotorula* deficient for para-aminobenzoic acid Science 100 85

ROBBINS W J & R MA 1945 Growth factors for *Trichophyton mentagrophytes* Am J Botany 32 509

SULZBERGER M B 1932 Experiments in passive transference of urticarial hypersensitiveness to fungous extracts J Immunol 23 73

test to immune serum but in whom there was a negative skin test to vaccine and a negative agglutination test In this patient, it was thought that excess antigen produced by *Candida albicans* masked the skin test and inhibited antibody formation In these two instances therefore an understanding of the patient s immune response was necessary for successful treatment Information concerning the significance of sensitivity and immune responses in other types of systemic fungus infections may prove equally as important

While the allergic and immune status of an individual patient should be studied with all of the available techniques it is also of great importance to establish the rate of infectic [illegible]
for a better understanding [illegible]
systemic fungus infections [illegible]
changed the concept of these diseases Both were thought to be highly fatal infections until skin test materials were developed that could detect minimal or past infections by eliciting a positive skin test comparable to and interpreted in the same way as the tuberculin test Gifford (1936) and Dickson (1937) were the first to demonstrate that a primary benign type of infection was caused by *Coccidioides immitis* Inhabitants in the known endemic areas of the disease have shown a high incidence of infection as determined by positive coccidioidin tests The secondary malignant progressive phase of coccidioidomycosis (coccidioidal granuloma) was found to occur infrequently and to be more prevalent in the dark skinned races

Until recently histoplasmosis has been considered a highly fatal infection Christie and Peterson (1945) Palmer (1945 and 1946) and Furcolow, High and Allen (1946) however have shown that in certain parts of the country, there is a high correlation between pulmonary calcification in nontuberculin reactors and positive skin tests to histoplasmin If this skin testing material proves to be specific (Emmons Olson and Eldridge 1945 Howell 1947) it will have shown that *Histoplasma capsulatum* also causes a primary benign type of infection and that the highly fatal form of the disease represents only a not too frequent secondary malignant type of infection

It is hoped that the future development of mycological methods will stress these physiological chemical immunological and epidemiological approaches and will yield a better understanding of the human pathogenic fungi Much has already been accomplished and the methods of study have been indicated Such a program will need the help of adequately trained personnel The personnel necessary for effective work would include a mycologist a physiologist an immunochemist and a pathologist This group should be in contact with clinical facilities that would provide cases for study and should be in contact with clinicians who would help select the problems to be investigated

Bibliography

BERNHEIM F 1942 The effect of various substances on the oxygen uptake of *Blastomyces dermatitidis* J Bacteriol 44 533
CHRISTIE A & J C PETERSON 1945 Pulmonary calcification in negative reactors to tuberculin Am J Pub Health 35 1131

Cormia F E & G M Lewis 1946 Experimental aspects of pen cill n sensitization with special reference to conjoint sensitization to superficial fungus diseases J Invest Dermat 7 375

DeLamater E D 1941 Experimental studies with the dermatophytes III Development and durat on of immunity and hypersensitivity in guinea pigs J Invest. Dermat 4 143

DeLamater E D & R W Benham 1938 Experimental stud es with the dermatophytes I Primary disease in laboratory animals J Invest Dermat 1 451

DeLamater E D & R W Benham 1938 Experimental stud es with the dermatophytes II Immunity and hypersensitivity produced in laboratory animals J Invest. Dermat 1 469

Dickson E C 1937 Valley fever of San Joaqu n Valley and fungus *Coccid oides* Calif & West Med 47 151

Dickson E ... rch Int Med 59 1029

Emmons C ... Stud es of the role of fungi in pulmona ... Pub Health Rep 60 1383

Furcolow ... Some epidem ological aspects of sensitivity to histoplasmin and tuberculin Pub Health Rep 61 1132

Gifford M A 1936 San Joaquin fever Ann Rep Kern County Health Dept 22 23

Hassid W Z E E Baker & R M McCready 1943 An immunologically active polysaccharide produced by *Coccidioides immitis* Rixford and Gilchrist J Biol Chem 149 303

Ha

Hia

Hir

Hirsch E F & D D Andrea 1927 The specific substance of *Coccidioides immitis* J Infect Dis 40 634

Howell A Jr 1947 Studies of fungus antigens I Quantitative studies of cross reactions between histoplasmin and blastomycin in guinea pigs Pub Health Rep 62 631

Jadassohn W F Schaaf & G Wohler 1937 Analyses of composite antigens by the Schultz Dale technic further experimental analyses of trichophytins J Immunol 32 203

Martin D S & D T Smith 1939 Blastomycosis (American blastomycosis Gilchrist s disease) I Review of literature II Report of thirteen new cases Am Rev Tuberc 39 275 488

Martin D S & R R Jones 1941 Systemic blastomycosis Report of a case with unusual immunologic findings well two years after onset Surgery 10 939

Nickerson W J 1946 Inhibition of fungus respiration a metabolic bio-assay method Science 103 484

Nickerson W J & J B Chadwick 1946 On respiration of dermatophytes Arch Biochem 10 81

Palmer, C E 1945 Nontuberculous pulmonary calcification and sensitivity to histoplasmin ...

Palmer C E ... dent nurses

Peck R L D ... dermatitidis

Peck S M & W ... The production of an antibiotic substance similar to penicillin by pathogenic fungi (dermatophytes) Pub Health Rep 60 148

Peck S M & S Siegal 1947 Immunologic relationships of the antibiotics and trichophytin Clinical observations and animal experiments J Invest Dermat 9 165

Robbins W J & R Ma 1942 Vitamin deficiencies of *Trichophyton discoides* Bull Torrey Bot Club 69 509

Robbins W J & R Ma 1944 A *Rhodotorula* deficient for para aminobenzoic acid Science 100 85

Robbins W J & R Ma 1945 Growth factors for *Trichophyton mentagrophytes* Am J Botany 32 509

Sulzberger M B 1932 Experiments in passive transference of urticarial hypersensitiveness to fungous extracts J Immunol 23 73

SUPERFICIAL DERMATOMYCOSES CAUSED BY *TRICHOPHYTONS, MICROSPORUMS,* AND *EPIDERMOPHYTONS*

By Fred D Weidman
Department of Dermatology and Syphilology School of Medicine, University of Pennsylvania Philadelphia Pa

If one were to consult a standard text on dermatology,[1] he would find such a long list of superficial fungus diseases caused by *Trichophytons Microsporums* and *Epidermophytons* that he would realize at once the impossibility of making a comprehensive presentation, even in a monograph like this I have decided, therefore, to steer a course which will be panoramic hoping to inform the general scientist and general pathologist, even at the expense of the dermatologist and skilled mycologist In so doing I will follow the avenue of the general pathologist and attempt to trace the natural history of the diseases in the premises

Accordingly this panorama first, will confine itself to dermatomycoses that are superficial (ringworm and favus) Second it will deal with the pathology of the group as a whole In connection therewith, certain features will be pointed out that either have been underemphasized or have some special application to medical biology at large To a large extent these special points have a bearing upon diagnosis and treatment, which are so important to the dermatologist

Habitat of the Fungi on Vegetation and Animals I am not aware of authenticated evidence that any of the three genera of fungi cited in the title live and grow upon vegetation in nature Members of all of them however frequently parasitize the skin of lower animals cats dogs horses cattle sheep monkeys and others[2] In short the diseases that they produce can be contracted from lower animals, but, at the same time it is thoroughly established that most of them are contracted from man to man Details in respect to animals can be found in the recent volume by Hull[3] Kadisch[4] has reviewed the subject in general

Transmission This is a story that is too extensive and complex to elaborate in detail here Suffice it to point out that one is dealing with fungous cells which live upon the surface of an animal or of man and which need only come in contact with the appropriate (receptive) soil on the surface of a human being Moreover after being shed from the animal (including man) the spores can lie dormant and viable in the environment for as much as three months in the case of *Trichophyton mentagrophytes*[6, 7] and twelve months for *Microsporum felineum*[8]

Predisposing Factors for Infection Considering the ubiquity of superficial fungus disease and the readiness with which the infectious material can be dispersed into the environment it is clear that many human skins are immune to infection Otherwise practically all human beings would become infected There is ample evidence of the close and continued contact of infected skins with healthy ones Many wives are free from dermatophytosis of the toes (athlete s foot) whereas their husbands toes are

notably diseased Again, in a family of ten, only one or two members will have favus—a favus, moreover, that is of several years' duration The remaining eight must be immune Natural immunity accounts for many of these cases, but certain investigations[8] make it appear that a high pH of the cutaneous surface plays a role in preventing infection Acquired immunity is indicated in the skin at a pre-existing patch of favus which has been found immune against experimental reinoculation for up to three months[9] Singularly, the endocrine system plays a role in tinea capitis Witness the fact that the infection can disappear spontaneously at puberty and can be made to disappear under treatment by stilbesterol In short, there is an outstandingly large factor of "the soil" in connection with that successful invasion of the skin which we call infection

Moisture, especially from sweating, is conducive to infection, especially in intertriginous places

The Tissue Reaction This is the morbid anatomy" of the general pathologist The range of tissue reaction is variable, *but is confined to the skin* Singularly, the disease does not extend to the internal organs Even when fungus spores are forcibly rubbed into denuded skin and reach internal organs, they survive only a few hours[10] It is assumed that the fungus perishes for lack of oxygen

In respect to the tissue reaction of the skin, the simplest is that in which only the epidermis participates That is, an inflammatory infiltration does not develop in the corium This simple reaction is not represented in any of the diseases caused by *Trichophyton, Microsporum,* and *Epidermophyton* It is represented in tinea versicolor Here, little more than a moderate hyperplasia of epidermal cells and hyperpigmentation take place The hyperplasia results in excessive keratinization, and the end result is a macule which is more or less brown, slightly scaly and scarcely congested Incidentally, the disease, not uncommonly, has a predilection for the orifices of the hair follicles, thus resulting in a stippled patterning On the face of it, this would speak for the presence of nutritive materials which originated in the pilosebaceous apparatus, but the matter could be much more complex than this In any event, there is a hint here toward the investigation of factors which might favor the growth of this fungus species

All three genera are concerned in the next higher level of tissue reaction such as is exemplified in ringworm of the scalp (tinea circinata) and dermatophytosis Here, it is definitely inflammatory The story is the conventional one in respect to the connective tissue reaction, namely, congestion and exudation The congestion is reflected in the redness observed clinically The exudation may mount to such proportions as to produce a vesicle or even a bulla In one form of ringworm of the scalp (kerion), it is so severe as to result in suppuration The epidermis participates in the form of a cellular hyperplasia (which the dermatologist denominates 'acanthosis') together with edema The fungus is confined to the epidermis In the case of vesicles and bullae, it is most abundant on the underside of the ceiling of the vesicle, and a practical suggestion therefrom is that the dermatologist should point toward this region when he is examining mate-

rial microscopically for fungus for purposes of diagnosis That is the bottom of the ceiling should be arranged upward on the microscopic slide Thereby, the fungus will not be obscured to the extent that it would be otherwise

Depending upon the individual case then the lesion either will be simply congested and hyperkeratotic (scaly) as in the simpler cases or will have vesiculation and bulla formation added as in severe ones Secondary infection accounts for the presence of pus in both vesicles and bullae

Secondary effects are well established in the form of ascending lymphangitis and cellulitis In addition certain claims have been made that dermatophytosis is responsible for thromboangiitis obliterans but it is too soon as yet to accept this theory as proven [3]

Mycotic Folliculitis (Ringworm and Favus) Here the infection extends more deeply below the surface *i e* into the deepest parts of the hair follicle (tinea capitis tinea barbae, and favus) With the exception of favus the fungus still invades only the epidermis in principle if one recalls that the sheath of the follicle and its contained hair shaft are but invaginations of surface epithelium The fungus gains entry at the follicular orifice after which it proceeds downward through the hair shaft It induces degeneration of the shaft which results in its loosening from the hair papilla The hair falls out or s broken off resulting in baldness Fortunately the papilla itself is not destroyed except in favus and after the d sease has regressed it resumes the formation of hair shafts The baldness is only temporary In infections with *Microsporum felineum* the reaction is so intense that suppuration ensues *i e* suppurative folliculitis This is the kerion of the dermatologist

hich extends be
It is the cause
which the hair
shaft grows with the result that permanent baldness results The damage that is produced in the connective tissues results in scarring This *Trichophyton* is the only member of the three genera under discussion which produces permanent damage to the skin

Immunity In spite of the extremely superficial diseases produced by these three fungus genera their products diffuse suffic into the corium to act as antigens This results in allergic tissue r more or less distant parts of the skin which are known as derma Naturally such lesions do not cont s substance The tion (lesion) is so highly varied tha e described in de Suffice it to say that the lesions ar ted type and di to imitate those of other dermatos hat even the m a is commonly baffled by o the usefulne og t ons for s of dia ugh to say t eous o *osis* nciples of the ally d ffe be ells an

larger and characteristic that resort need not be had as frequently to biologic tests. Commonly, the morphology in the test tube or under the microscope suffices. For details, see the text by Lewis and Hopper.[1] Incidentally, ultraviolet light (Wood's light), played upon a fungus colony, induces differences in color which have diagnostic value, but the usefulness of this method is impaired because much skill is entailed in the reading of the colors. The assistance of a skilled mycologist is required here. Such a light, however, has come into general use clinically. When directed upon a lesion the fungus-infected materials fluoresce spectacularly. The species of fungus is not indicated in such examinations. Only the fact that the disease is mycotic is demonstrated.

Treatment. As in the past, fungicidal chemicals are the main reliance today, together with X ray epilation in the case of ringworm of the scalp.[13] One notable advance has been made by discovering fungicidal value in certain fatty acids. These have the advantage that they do not induce untoward inflammatory reactions. Hopkins and his co-workers[14] investigated them in respect to dermatophytosis, employing volunteers at Army establishments during World War II, and fixed upon propionates and undecylenates. Peck and Russ[15] prefer propionate-caprylic mixtures. The entire subject, including the requirements for fungicide testing, has been reviewed recently.[16]

In the treatment of ringworm of the scalp (tinea capitis) it has been discovered recently that fungicidal applications suffice to cure infections by *Microsporum felineum*, whereas infections by *Microsporum audouini* remain resistant and require X ray treatment. This has lead to increased demands upon the laboratory for the identification of the fungus species in the case.

In any event, many of the ringworm infections are resistant to cure even under the best circumstances. This has lead to praiseworthy attempts in other directions than the stereotyped fungicidal chemicals. Thus Chambers and Weidman[17] discovered a fungistatic strain of *Bacillus subtilis* in normal toes which inhibited a pathogenic fungus of ringworm of the toes. That is, antibiotic therapy was essayed. Lewis[18] has continued such studies but the results have not been sufficiently proven.

Loth[19] secured good results in the treatment of ringworm of the scalp by the administration of stilbesterol by mouth, but results have not been sufficiently uniform in other hands to establish this method. There is a logical basis for this form of treatment, because it is an established fact that tinea capitis disappears spontaneously at puberty and is almost unknown after that time.

Dolce and Nickerson[20] employed zinc chloride locally on the basis that it might inhibit respiration of the fungus. Although they failed, it is nevertheless true, as they pointed out, that it is the avenues directed against the biology of the fungus that should be followed in future investigations. Only in this way can one expect to emerge from the welter of stereotyped empirically selected chemical fungicides and uncover substances that are epoch making in therapy.

Bibliography

1 SUTTON R [illegible] Edition. Mosby S [illegible] es of the Skin [illegible] seases of the Skin [illegible] 1948 Introductio [illegible]

2 HELLER J [illegible] Berlin JADASSOHN [illegible]

3 HULL, T G [illegible] tion Thomas [illegible]

4 KADISCH F 1923 *The occurrence of pathogenic fungi outside the body* Dermat. Wchnschr Leipsic 89 (40) 1423

5 GLASS F A 1948 Viability of fungus in hairs from patients with tinea capitis. I *Microsporon audouini* Arch D & S 57 122

6 FARLEY D L 1921 Viability of ringworm fungi in dry cutaneous material Arch D & S 3 759

7 WEIDMAN F D 1927 The laboratory aspects of dermatophytosis Arch D & S 15 415

8 PECK S M H ROSENFELD W LEIFER & W BIERMAN 1939 Role of sweat as fungicide with special reference to use of sweat in therapy of fungous infections. Arch Derm & Syph 39 126-148

9 GREENBAUM S S 1924 Immunity in ringworm infections Arch D & S 10 279

10 SULZBERGER M 1928. The pathogenesis of trichophytids Arch D & S 18 891

11 REISS F & J B GRAHAM 1946 Absence of thromboangiitis obliterans in experi [illegible]

12 [illegible] Arch D & S 45 1113

13 [illegible] 1946 Local treatment of tinea [illegible]

14 [illegible] LEDIN & G REBELL. 1944 Treatment and prevention of dermatophytosis and related conditions Bull U S Army Med Dept 77 42

15 PECK S M & W R RUSS 1947 Propionate-caprylic mixtures in the treatment [illegible]

16 [illegible] G M LEWIS 1945 The war [illegible]

17 [illegible] static strain of *Bacillus subtilis* [illegible]

18 [illegible] *In vitro* fungistasis by a bac [illegible]) & S 54 300

19 [illegible] erapy in tinea capitis Arch. D & S 48 121

20 DOLCE F A & W J NICKERSON 1947 Treatment of mycotic infections by inhibiting respiration of dermatophytes Arch D & S 55 379

CHROMOBLASTOMYCOSIS*

By Arturo L. Carrión
School of Tropical Medicine San Juan, Puerto Rico

Clinical Aspects

Definition Chromoblastomycosis is a chronic, infectious, apparently noncontagious skin disease confined most frequently to one of the lower extremities and characterized clinically by the formation of nodular, verrucous, or tumorlike lesions The infection may be caused by any of several species of dematiaceous fungi

Synonymy The term "chromoblastomycosis," first used by Terra *et al*[2] in 1922 is not only a long word, but a misnomer falsely ascribing the disease to a *Blastomyces* and conveying the erroneous impression of an extraordinary color element in the clinical picture Notwithstanding this, we believe that, for the present, this name should be preserved It has been extensively used in the literature and it expresses the frequent resemblance of the disease to true blastomycosis Furthermore, the prefix "chromo," though misleading in regard to the clinical picture, is descriptive of the etiologic fungus

The following synonyms have been used or suggested for this disease: *Blastomycose negra* (Pedroso[3]), *figueira* (Rudolph[4]), *dermatite verrucosa por Phialophora verrucosa* (Pedroso and Gomes[5]), *formigueiro* (Gomes[6]), chromomycosis (Moore and Almeida[7]), *dermatite verrucosa cromomicósica* (Redielli[8]) *dermatitis verrucosa blastomicótica* (Boggino[9]), Pedroso's disease, Fonseca's disease, or Gomes' disease (Weidman and Rosenthal[10]), Pedroso and Carrión's disease (Barros Barreto[11]) Some of the synonyms just mentioned are almost as long or longer than chromoblastomycosis and are still misnomers, others might be criticized for bearing personal names If the term "chromoblastomycosis" were to be changed at all, it should be done at an international congress, where the subject could be thoroughly discussed and settled by general agreement

History In 1911, Pedroso, of São Paulo, Brazil, noted the presence of large dark brown to yellowish, spherical bodies in a biopsy from a patient with nodular and ulcerated skin lesions of the foot and leg (Pedroso and Gomes[5]) He suspected a mycotic infection and was able to isolate from the lesions a dark-colored fungus The disease became known as *blastomycose negra* in Pedroso's laboratory (Pedroso[3]) but the study of the presumptive causative agent was postponed and the discovery was not reported until 1920[8]

In 1914, Rudolph[4] published his observations on a skin disease popularly known as *figueira* in Minas Geraes and Goyaz The clinical description and mycologic findings given by Rudolph in his report clearly indicate that he was dealing with the same disease observed by Pedroso in São Paulo three years before

* In the preparation of this paper, the author has partly transcribed and partly abstracted the information contained in a chapter on chromoblastomycosis previously prepared for "Biology of Pathogenic Fungi" (Annales Cryptogamici et Phytopathologici) by Carrión and Silva. Some additional information has also been included

In 1915, Medlar[12] and Lane[13] in the United States published their observations on a cutaneous infection caused by a new fungus ' in a patient from Boston The lesions of this patient were of blastomycetic type and contained numerous spherical pigmented parasitic cells The etiologic fungus was carefully studied and named *Phialophora verrucosa* From the descriptions of Medlar and Lane, it was apparent that the disease in the Boston patient was identical in nature with that previously observed in Brazilian patients by Pedroso[5] and Rudolph[4]

In 1922, Brumpt[14] established that the organism causing the infection in Pedroso s original case was not *Phialophora verrucosa* but a new species which he named *Hormodendrum Pedrosoi* Subsequent studies on the morphology of the latter fungus have led to one of the most interesting chapters in the history of chromoblastomycosis In 1923, Fonseca and Leao[15] described for this fungus a second method of sporulation which corresponds closely with the genus *Acrotheca* In 1935, it was found in our laboratories[16] that the species *Pedrosoi* possesses still another method of sporulation by which the conidia are produced in phialides, thus establishing its relationship to a third form genus namely, *Phialophora Medlar*, 1915[17] The simultaneous occurrence of three methods of sporulation in one and the same organism and the various proportions in which these methods may be represented in different isolates of the parasite led to the description of the species under different specific and generic names The literature on the subject became extremely confusing However, after years of painstaking work in our laboratories[18,19] the synonymy of these names became firmly established and the binomial *Fonsecaeae Pedrosoi*, proposed by Negroni[20] of Argentina has been accepted as the most convenient name until the perfect form of the fungus becomes known

In 1935 we described in a Puerto Rican case, a third etiologic agent for chromoblastomycosis The Puerto Rican fungus reveals the three methods of sporulation characteristic of the genus *Fonsecaea*, but it possesses sufficiently distinct characteristics to warrant its registration as a new species which bears the name *Fonsecaea compactum*[21,22]

According to the above observations it is evident that chromoblastomycosis like mycetoma may be caused by many different fungus parasites In addition to the three etiologic fungi just reviewed, a few others which appear to be legitimate species have been recently described Although the latter species are not generally recognized, they are worthy of consideration and will be discussed later under ' Mycologic Aspects "

Incidence and Geographic Distribution The existence of chromoblastomycosis has been established in North, South, and Central America, the West Indies Europe Africa, the East Indies, Japan, and Australia, leaving continental Asia as the only part of the world as yet without reported cases Among the infections classed as chromoblastomycosis, there are 159 in which the diagnosis appears to have been made on a sound basis and this number might be accepted as an approximate index of the recognized cases up to the year 1947 These cases are distributed as follows Cuba 43 Brazil 41 Puerto Rico, 15*, South Africa, 12 United States 9

* Among the 15 Puerto Rican cases, there are 8 which have not been officially published yet.

Venezuela, 9, Russia, 5, Costa Rica, 4, Dutch East Indies, 4, Japan, 3, Algiers, Argentina, and Australia, each, 2, and Rhodesia, Dominican Republic, Guatemala, Canal Zone, Mexico, Canada, Paraguay, and Uruguay,

chromoblastomycosis might be considered as a cosmopolitan disease, but its distribution would be predominantly tropical

Symptomatology The disease usually affects one of the lower extremities It begins as a small papule or warty growth, which may develop anywhere on the extremity, but is located, as a rule, on some part of the foot, whence the infection spreads upward through the gradual development of satellite lesions The course of the pathologic process is slow, and the



FIGURE 1 A Puerto Rican case of chromoblastomycosis of fifteen years duration The lesions occurred in great numbers especially toward the distal portion of the extremity and they were conspicuously varied in morphology

clinical history often reveals that the infection has existed for ten or more years at the time of examination

In a typical well-advanced case of chromoblastomycosis, the foot and leg are generally swollen and somewhat elephantiasic in appearance (FIGURE 1) The lesions occur in great numbers, especially toward the distal portion of the extremity, and they are conspicuously varied in morphology For the purpose of description it is convenient to class them in five different types, namely the nodular the tumorous, the verrucous, the plaque, and the cicatricial

The nodular type includes the youngest and smallest elements in the clinical picture and consists of moderately elevated fairly soft, dull pink to violaceous growths, the surface of which may be smooth, verrucous or scaly

Through further development, many nodules are gradually transformed into lesions of the second, or tumorous type This type is represented by much larger and more prominent, distinctly papillomatous, sometimes

lobulated, tumorlike masses, partly or wholly covered with dirty gray epidermal débris crusts, and horny particles. On the foot and lower leg, where the pathologic process tends to be most exuberant, the tumor masses often reach enormous dimensions, taking on the characteristic appearance of cauliflowers.

In the third, or verrucous, type of lesion, hyperkeratosis is the outstanding feature, the efflorescences are warty in appearance and may resemble verruca vulgaris. Growths of the verrucous type are frequently encountered along the borders of the foot.

The plaque type is the least common of the lesions of chromoblastomycosis. It consists of fairly flat, slightly elevated, variously sized and shaped areas of infiltration. They are reddish to violaceous in color, superficially scaly, and some of them show exaggeration of the lines of cleavage. The development of small, papillomatous vegetations, or larger nodules, within a plaque is sometimes observed and may lead to great variations in the morphology. When present in the clinical picture, the plaques are generally found on the higher portions of the extremity and never on the lower leg or foot.

Finally, the cicatricial type of lesion is represented by growths that enlarge by peripheral extension, while healing takes place at the center with the production of sclerotic or atrophic scarring. Cicatricial lesions may cover more or less extensive areas and are usually annular, arciform or serpiginous in contour.

The lesions of chromoblastomycosis develop slowly but progressively, and, in the course of time a few or many of them may coalesce to form extensive and often bizarre aggregates. The infected tissues are easily traumatized and bleed readily. When pressed with the fingers many of the lesions discharge a whitish caseous material and sometimes fluid pus at one or more points. Secondary bacterial infections and ulceration frequently complicate the clinical picture and are mostly responsible for the foul smelling character of the eruption a feature that is usually perceptible at a distance from the patient. Subjectively, pruritus may be an important symptom and some patients complain of pain. In advanced cases, there is partial or total incapacity for work.

The deeper tissues are not usually involved. The lymphatic glands draining the diseased focus may participate in the process, but this is not the rule. However, adenitis due to bacterial complications is not infrequent. Metastases through the blood stream appear to be extremely rare, but there is no question that they can be produced (Carrión and Koppisch[illegible]). Finally, no systemic symptoms have yet been recorded from the infection.

It should be emphasized that the dermatologic picture just given is a general representation of the symptomatology in well developed typical cases of chromoblastomycosis of the lower extremities. Although location in one of the lower extremities is the rule, the disease may affect the skin in almost any other part of the body, either as aberrant lesions produced during the course of a limb infection or as the original site of attack. Involvement of the upper extremities ranks second in order of frequency, the initial lesion usually being located somewhere on the hand or wrist, although it may ap-

been variously described as ochre, olivaceous, yellowish-green, and dark chestnut The parasitic cells may occur singly or in groups In the cutis they are variously located within giant cells, free in the tissues, and, not infrequently, in the center of microabscesses, but they may be found also within epithelial pearls and microabscesses in the epidermal layers Evidence of germination is often noted in the *stratum corneum*

Etiologic Factors Chromoblastomycosis occurs most frequently during the period of active adult life Among 109 authentic cases of the disease



FIGURE 2 Histopathologic section of a lesion in chromoblastomycosis The epidermis is thickened and folded to fit the underlying papillomatous elevations In the cutis the reaction is essentially granulomatous, with a varied cellular infiltrate

in which the age was recorded, 77 (nearly 71 per cent) lay between the ages of 20 and 50 years The extreme ages at which infection took place were 3 (Tschernjawski[28]) and 76 (Takahashi[29]) years, respectively

Chromoblastomycosis is decidedly more common among males A review of 138 authentic cases in which the sex was registered showed that 132 were males, a proportion of 96 per cent

There seems to be no race immunity However, in a collection of 124 clinical histories containing data regarding the race of the patients, it was

(all of them from Russia) Africa 4 isolates (1 from Algiers and 3 from the Union of South Africa) Asia 1 isolate from Japan, and the East Indies 2 isolates (1 from Java and 1 from Sumatra)

Morphology in Pathologic Tissue Since chromoblastomycosis in human beings is essentially a skin disease, the infecting fungus is to be found almost exclusively in cutaneous lesions Here it may be recognized in both the dermis and epidermis as characteristic usually spherical occasionally crescent shaped brownish yellow bodies so called sclerotic cells measuring about 10 microns in diameter (FIGURES 4 and 5) These bodies may occur either singly or in clumps rarely in short chains They are found within giant cells free in the tissues in the center of microabscesses or enclosed within epithelial pearls in the Malpighian and horny layers of the epidermis

The fungus elements possess a dark, fairly thick cell wall part of which is occasionally swollen, bulging toward the interior of the cell The wall is sometimes covered with a crusty layer of refractile material (FIGURE 6a) The protoplasm is granular, contains refractile inclusions, and possesses a

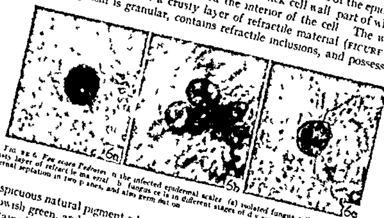


FIGURE 6. *Fonsecaea Pedrosoi* in the infected epidermal scales (a) isolated fungus cell covered with a crusty layer of refractile material (b) fungus cells in different stages of division (c) fungus cell showing internal septation in two planes, and also germination

conspicuous natural pigment which has been described as ochre olivaceous yellowish green, and chestnut brown The nucleus is not apparent in fresh or stained preparations Germination of the parasitic cells is frequently observed in the *stratum corneum* of the epidermis (FIGURE 6c) In the infected tissue multiplication of the fungus takes place by fission different stages of the process being noticeable in both the dermis and epidermis (FIGURE 6b)

Gross Morphology in Culture On Sabouraud's *milieu d'epreuve* and on 4 per cent dextrose agar after the fourth week and at room temperature cultures resemble flattened cones, measuring about 3 5 cm in diameter (FIGURE 7), center of culture is elevated about 8 mm above medium sometimes forming mammillary prominence rest of culture gently slopes often radially the aerial mycelium forms dark gray greenish olivaceous gray or brownish, feltlike network On Czapek's agar colonies are poorly developed mycelium is mostly submerged gray to olivaceous forming at times fluorescent outgrowths central zone shows shallow layer of aerial

Morphology in Culture The microscopic characters are usu

Fonsecaea compactum, *Phialophora verrucosa*, *Torula poikilospora*, and two *Hormodendrum* species described respectively by Simson[3] and O'Daly.[8] The second group consists of four organisms which produce primarily soft, dark moist colonies undergoing what appears to be a yeast like phase in the course of their development. As the cultures age, the thallus becomes partly filamentous, but the growth as a whole retains its moist appearance. Only two of the organisms of this group have been published: *Hormiscium dermatitidis*, by Kano[31] and a "black *Candida* like species," described by Berger.[35] Of the two remaining fungi of this group, one was isolated by C. Bonne in the East Indies, and the other was obtained by us in Puerto Rico. The descriptions of these two fungi are not yet available.

Fonsecaea Pedrosoi. Authorship: Brumpt, 1922[14], Negroni, 1936 *comb nov*.[20] Carrion, 1940 *emend*.[15]

Synonymy: *Hormodendrum Pedrosoi* (Brumpt[14]), *Phialophora verrucosa* (Pedroso and Gomes[5]), *Acrotheca Pedrosoi* (Fonseca and Leao[18]), *Hormodendrum algeriensis* (Montpellier and Catanei[36]), *Acrotheca Pedrosiana* (Bonne[37]), *Trichosporium Pedrosianum* (Ota[38]), *Acrotheca verrucosa* (Tschernjawski[39]), *Trichosporium Pedrosoi* (Langeron[39]), *Hormodendrum rossicum** (Merlin[40]), *Cladosporium algeriensis* (Vuillemin[41]), *Gomphinaria Pedrosoi* (Dodge[42]), *Hormodendroides Pedrosoi* (Moore and Almeida[43]), *Phialophora macrospora* (Moore an[illegible] [illegible] and Almedia[43]), *Phialoconidi*[illegible] *dendrum japonicum** *Carrionia Pedrosoi* (Briceno Irigaorri[46]), *Hormodendrum chaquense*† (Mazza and Niño[47]), *Phialophora Pedrosoi* (Binford *et al.*[48])

Geographic Distribution: *Fonsecaea Pedrosoi* has never been found in nature outside of the human body, but the widespread distribution of its victims has established its ubiquity. It is the most common etiologic agent of chromoblastomycosis. Among 90 legitimately classified organisms isolated from the disease throughout the world, 76, or 84 per cent, belong to this species. The geographic distribution of the fungus is conspicuously higher in the warmer climates. This is evident from the fact that 63, or 83 per cent, of the 76 isolates just mentioned, were obtained from patients who contracted the infection in tropical or subtropical regions.

The continental distribution of *Fonsecaea Pedrosoi* has been as follows: North America, 5 isolates, (4 from the United States and 1 from Mexico); Central America, 4 isolates, (3 from the Panama Canal Zone‡ and 1 from Guatemala), South America, 25 isolates, (19 from Brazil, 4 from Venezuela and 2 from Argentina), West Indies, 31 isolates, (18 from Cuba, 11 from Puerto Rico,§ and 2 from the Dominican Republic||), Europe, 4 isolates [illegible]

[illegible]

|| Of the two isolates from the Dominican Republic only one has appeared in the literature as [illegible] know (Carrión and Pimentel Imbert[3].) The other was studied by the author due to the courtesy of M. F. Pimentel Imbert.

(all of them from Russia) Africa 4 isolates, (1 from Algiers and 3 from the Union of South Africa), Asia, 1 isolate from Japan, and the East Indies, 2

dermis and epidermis as characteristic, usually spherical, occasionally crescent shaped, brownish yellow bodies so-called "sclerotic cells" measuring about 10 microns in diameter (FIGURES 4 and 5) These bodies may occur either singly or in clumps, rarely in short chains They are found within giant cells, free in the tissues, in the center of microabscesses, or enclosed

The protoplasm is granular, contains refractile inclusions, and possesses a



FIGURE 6 *Fonsecaea Pedrosoi* in the infected epidermal scales (a) isolated fungus cell covered with a ... layer of refractile material (b) fungus cells in different stages of division (c) fungus cell showing ... septation in two planes and germination

conspicuous natural pigment which has been described as ochre, olivaceous, yellowish green, and chestnut brown The nucleus is not apparent in fresh or stained preparations Germination of the parasitic cells is frequently observed in the *stratum corneum* of the epidermis (FIGURE 6c) In the infected tissue multiplication of the fungus takes place by fission different stages of the process being noticeable in both the dermis and epidermis (FIGURE 6b)

Gross Morphology in Culture On Sabouraud's *milieu d'épreuve* and on 4 per cent dextrose agar after the fourth week and at room temperature cultures resemble flattened cones, measuring about 5.5 cm in diameter (FIGURE 7) center of culture is elevated about 5 mm above medium, ... forming mammillary prominence rest of culture gently slopes ... folded the aerial mycelium forms dark gray greenish olivaceous ..., or dark brownish felt-like network On Czapek's agar colonies ... developed mycelium is mostly submerged gray to olivaceous, ... border arborescent outgrowths, central zone shows shallow ... aerial hyphae

Microscopic Morphology in Culture The microscopic characters ...

ally more conspicuous after the third week of growth The vegetative hyphae are long, straight or undulated 1 25 to 3 microns in diameter, septate, branching cell walls thick and dark, protoplasm olivaceous, granular, with refractile droplets

The sporulation is of three different types, namely, the *Hormodendrum*, the *Fonsecaea*, and the *Phialophora* types These methods of sporulation may be occasionally combined in the same spore head The *Hormodendrum*

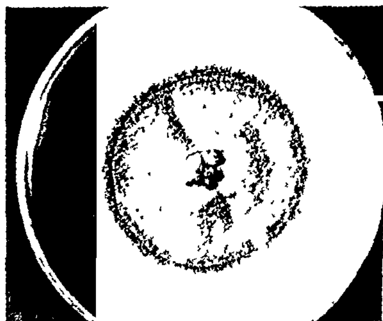
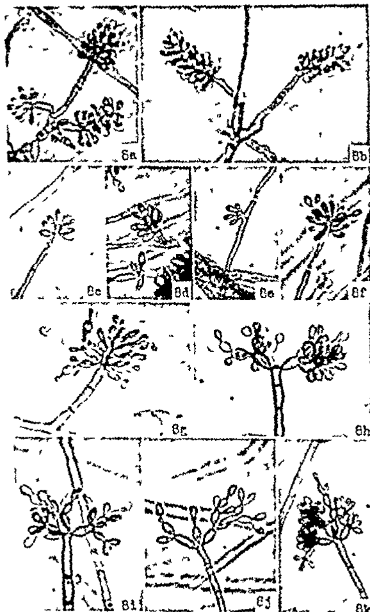


FIGURE 7 *Fonsecaea Pedrosoi* culture four weeks old developed at room temperature on Sabouraud's m[illegible] d eprew e

branches are erect or ascending, with terminal cell or conidiophore sometimes darker having at the tip several tiny, truncate, conical prominences to which the spores are attached The conidia are borne in chains, usually short which tend to branch by multiple budding at the distal pole of each successive conidia resulting in the formation of complicated spore heads The conidia are unicellular, ovoid often elongated, those at the base of the chain being frequently shield shaped and occasionally bicellular They measure 3 to 5 by 1 5 to 3 microns (basal elements 2 5 to 3 5 by 7 to 10 microns) are olivaceous in color, have smooth, thick, and dark walls and moderately to poorly developed disjunctors The *Fonsecaea* type of sporulation (FIGURES 8a to 8h) is more or less abundant, usually more conspicuous on corn meal agar cultures and sometimes predominating sometimes ob


8a
8b
8c
8d
8e
8f
8g
8h
8i
8j
8k

solete according to individual isolate The conidiophores are short or long straight or irregular exceptionally branched consisting of one sometimes more articles disposed terminally, laterally, or intercalarily, often derived from spore element in *Hormodendrum* head (FIGURE 8h) The surface of the conidiophore is wholly or partly verrucous due to the presence of tiny truncate, conical prominences to which the spores are attached, its pigmentation often is darker than vegetative mycelium The conidia are sometimes single (not catenate), forming clusters which may be indistinguishable when terminal from those of *Acrotheca* (FIGURES 8a 8b and 8c) some clus-

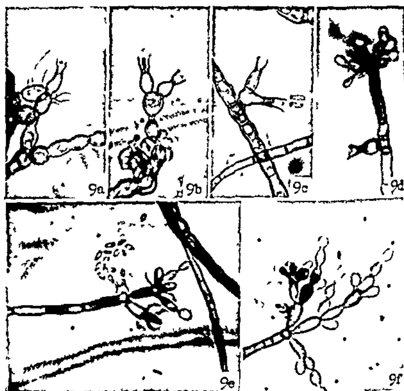


FIGURE 9 *Fonsecaea Pedrosoi* microscopic morphology representing the *Phialophora* type of sporulation Note association of the *Phialophora* and *Hormodendrum* types of sporulation in d e and f

ters showing tendency to chain formation as in *Hormodendrum* (FIGURES 8f and 8g) *The morphology of the conidia resembles terminal and sub*terminal elements of *Hormodendrum* heads already described The *Phialo-*

are

sparsely produced in culture

According to the various proportions in which the three methods of conidial formation just described may be represented in different isolates of

Pedrosoi, this species has been subdivided into four different varieties namely *Fonsecaea Pedrosoi* var *typicus* Carrion 1940[5] *Fonsecaea Pedrosoi* var *Cladosporioides* Carrion 1940[5] *Fonsecaea Pedrosoi* var *Phialophorica* Carrión 1942[19] and *Fonsecaea Pedrosoi* var *communis* Carrion 1940[5]. The latter is the most common variety encountered and its members always show an abundance of *Hormodendrum* and *Fonsecaea* sporulations. The *Phialophora* cups are sometimes as abundant as the other two types but usually they are scant.

Biologic Characters and Reactions It is generally admitted that *Fonsecaea Pedrosoi* requires an acid medium with a pH ranging between 5.5 and 6.5. The optimum temperature of this fungus has not been determined precisely but its mycelium develops profusely and sporulation is abundant at room temperature. A temperature of 100°C is lethal in 15 minutes while a temperature of 50°C is well tolerated for at least one hour. The fungus grows best under aerobic conditions. There is no doubt however that it may grow to a certain extent at reduced oxygen tensions. Although *Fonsecaea Pedrosoi* may be artificially stained by different laboratory methods it has shown no apparent reaction to Gram's method of staining. With the Ziehl Neelsen technique it takes on a darker and somewhat reddish color.

The biochemical activities of *Fonsecaea Pedrosoi* do not appear to be important. The fungus has been tested repeatedly for fermentative action against a large number of sugars with negative results. Other tests to determine its action on milk gelatin and serum are too few and conflicting to permit conclusions.

Immunologic Reactions Merin[4] claims to have induced specific positive intracutaneous tests in patients with chromoblastomycosis using as antigens culture extracts of *Fonsecaea Pedrosoi*. *Fonsecaea Pedrosoi* is also capable of inducing the production of specific complement fixing antibodies. This property has been determined in infected human beings by Baliña *et al* [4] and Martin *et al* [4] and in experimentally inoculated animals by Conant and Martin [4].

Response to Antibiotic and Chemical Agents (1) Alcohol. Experiments subjecting *Fonsecaea Pedrosoi* to the action of ethyl alcohol in different concentrations are being conducted in our laboratories. Although the observations are not yet completed it may be stated that 95 per cent alcohol is lethal to all the isolates in 3 minutes and to most of them in one or two minutes. (2) Sulfonamides. Keeney *et al* [8] observed that sodium sulfamerazine produced a pronounced inhibiting action against *Fonsecaea Pedrosoi*. The use of this drug in a few patients with chromoblastomycosis, however has not shown so far significant improvement. (3) Fatty acids. Keeney *et al* [8] have also studied the effect of fatty acids on the same group of fungi *in vitro* and have found that certain salts of these acids particularly sodium caprate and sodium undecylenate possess striking fungistatic and fungicidal action against *Fonsecaea Pedrosoi*. In view of the toxicity of these salts for the albino mouse these authors do not recommend the internal administration of such salts in the treatment of the deep mycoses of man until further animal experiments warrant their use. There is no

solete according to individual isolate The conidiophores are short or straight or irregular, exceptionally branched consisting of one some more, articles disposed terminally laterally or intercalarily, often d from spore element in *Hormodendrum* head (FIGURE 8h) The surf the conidiophore is wholly or partly verrucous, due to the presence truncate, conical prominences to which the spores are attached, its pigr tion often is darker than vegetative mycelium The conidia are sor single (not catenate), forming clusters which may be indisting when terminal from those of *Acrotheca* (FIGURES 8a 8b and 8c) s

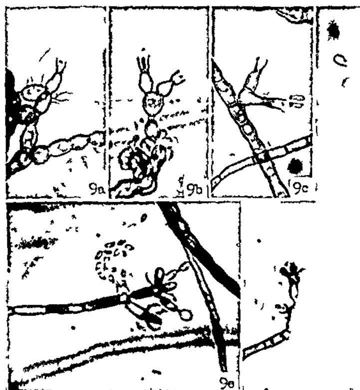


FIGURE 9 *Fonsecaea Ped osoi* m roscop c morphology represent n t on Note assoc at on of the *Ph alophora* and *Ho modend um* types o

ters showing tendency to chain formation as in 8f and 8g) The morphology of the conidia res terminal elements of *Hormodendrum* heads alread *phora* type of sporulation (FIGURE 9) is usually s dance its morphology is similar to that of *Phia*

Chlamydospores of the sclerotic cell type al sparsely produced in culture

According to the various proportions in which ial formation just described may be represent

sporulating ability of the fungus, (b) the outstanding and misleading predominance of one of the sporulating types in each of the varieties *typicus*, *Cladosporioides*, and *Phialophorica*, and (c) failure to recognize the specific identity of these three varieties as established by the existence of intergrading forms (variety *communis*), which link them together and preclude any subdivision of the group beyond the ranks of varieties. The classification of *Pedrosoi* as a *Fonsecaea* has been accepted here on the following basis (1) *Fonsecaea* Negroni, 1936 emend.,[11,18] is a legitimately created generic group including at least two well-differentiated species, namely, *Pedrosoi* and *compactum*, (2) it admits, *a priori*, the simultaneous occurrence of the

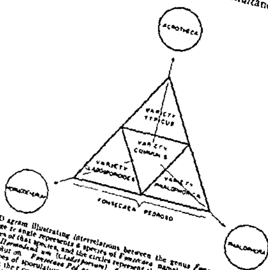


FIGURE 10. Diagram illustrating interrelations between the genus *Fonsecaea* and other related form genera. The large triangle represents a species of *Fonsecaea* namely *Pedrosoi*. The included smaller triangles are varieties of that species, and the circles represent the three genera to which *Pedrosoi* appears to be related, namely *Hormodendrum* (*Cladosporium*), *Acrotheca* and *Phialophora*. The arrows indicate suggested lines of evolution. *Fonsecaea Pedrosoi* var. *communis* (center of triangle) possesses in conspicuous abundance the types of sporulation characteristic of the three genera above mentioned and, thus, would appear to represent their common origin. Different trends of evolution probably led to the development of the varieties *Cladosporioides*, *typicus* and *Phialophorica*, each of which shows marked predominance of one of the types of sporulation, with equally marked reduction of the other two types. Further progress along the same line of evolution may have led to the differentiation of the three genera represented by circles, in which two of the sporulating types have disappeared completely, sporulation taking place in each group by a single method.

Hormodendrum, *Acrotheca*, and *Phialophora* methods of sporulation (FIGURE 10) as noted in its constituent species (3) it covers without strain the different varieties of *Pedrosoi*, (4) it leaves out fungi that are exclusively *Hormodendrum*, *Acrotheca*, or *Phialophora* in type (FIGURE 10), (5) it recognizes the existence of a characteristic *Fonsecaea* method of sporulation which is present in all the known strains of the group (FIGURE 8) reaching its highest degree of development in *Fonsecaea Pedrosoi typicus* (FIGURES 8a and 8b), (6) as a generic name it is neither misleading nor confusing and finally (7) *Fonsecaea* has already become extensively popular as noted in a review of recent publications on chromoblastomycosis.

In view of the fact that the species *Pedrosoi* is an imperfect fungus possess

reason, however, why these new drugs should not be tried locally on the cutaneous lesions of chromoblastomycosis (4) Penicillin The sensitivity of *Fonsecaea Pedrosoi* to penicillin has been tested by Keeney *et al*,[44] as well as by Fleming and Queen,[67] with negative results in both cases

Host Range Man is the only well known natural host susceptible to infection with *Fonsecaea Pedrosoi* It is possible that a spontaneous infection noted by Carini[58] in the lungs and kidneys of a batrachian (*Leptodactylus pentadactylus*) was produced by this or a closely related fungus Rudolph[4] observed in Brazilian cattle another disease similar to human chromoblastomycosis, but no information is given regarding the true nature of the pathologic process

Many investigators have endeavored to produce artificial infection with *Fonsecaea Pedrosoi* Takahashi (1937)[79] reported on the reproduction of [illegible]

[illegible]

cum) isolated from his patient The induced lesions were consistent histopathologically with chromoblastomycosis and the etiologic fungus could be recovered in culture

Attempts to produce artificial infection have been carried out on eight different species of experimental animals, namely, rats, mice, rabbits dogs monkeys guinea pigs, pigeons and frogs Successful infections have been claimed in all but the last two species mentioned From the experiments so far reported, it would seem that, among the different animals tested, the rat and the mouse are the species more susceptible to infection with *Fonsecaea Pedrosoi*, that infection may be effected by more than one route of inoculation and finally that the intraperitoneal, the subcutaneous, and the intratesticular are the most adequate routes to produce infection

Infection in laboratory animals may be more or less limited in extent or it may be systemic, or even fatal, according to the method of inoculation The lesions are nodular, variously sized, usually small, sometimes suppurating, single few or numerous, cutaneous, or internal (liver, lungs kidneys peritoneum, testicles), when cutaneous sometimes verrucous The histopathology represents a granulomatous type of tissue reaction similar to that noted in human lesions with the infecting parasite constituting a prominent part of the picture

Taxonomy Up to the present time, the species *Fonsecaea Pedrosoi* has shown no evidence of sexual reproduction and, consequently, it has its place among the Fungi Imperfecti In view of the general morphology, sporulating habits, and dark color of the parasite, it has been included in the order Moniliales, family Dematiaceae Finally, the simultaneous occurrence, in this organism, of three types of sporulation, which correspond to the genera *Hormodendrum Phialophora*, and *Acrotheca*, respectively, has led to the classification of the species in the new genus *Fonsecaea* Negroni, 1936[20] as emended by Carrion[18,19] *Fonsecaea Pedrosoi* has been subdivided into the four varieties *typicus*, *Cladosporioides*, *Phialophorica* and *communis*

The generic position of the "*Pedrosoi*" group has been a much debated subject Differences of opinion have arisen mainly from (a) the triple

sporulating ability of the fungus, (b) the outstanding and misleading predominance of one of the sporulating types in each of the varieties *typicus*, *Cladosporioides*, and *Phialophorica*, and (c) failure to recognize the specific identity of these three varieties as established by the existence of intergrading forms (variety *communis*), which link them together and preclude any subdivision of the group beyond the ranks of varieties. The classification of *Pedrosoi* as a *Fonsecaea* has been accepted here on the following basis (1) *Fonsecaea* Negroni, 1936 emend., is a legitimately created generic group including at least two well-differentiated species, namely, *Pedrosoi* and *compactum*, (2) it admits, *a priori*, the simultaneous occurrence of the

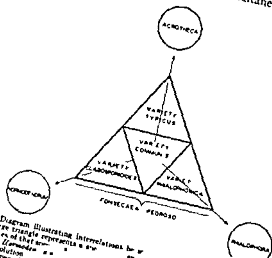


FIGURE 10 Diagram illustrating interrelations be[illegible] genera The large triangle represents a [illegible] ing are varieties of that [illegible] related, namely *Hormoden*[illegible] gested lines of evolution [illegible] abundance the types of sp[illegible] appear to represent the [illegible] the varieties *Cladosporioides* [illegible] of the types of sporulation w[illegible] the same line of evolution [illegible] which two of the sporulating types have disappeared completely [illegible] by a single method [illegible] u to the differentiation of the other two types [illegible] which shows marked predominance of one [illegible] ably led to the development of [illegible] au and fructious [illegible] thus, would [illegible] sporulation taking place in each group [illegible] Further progress along [illegible] genera represented by circles, in

Hormodendrum, *Acrotheca*, and *Phialophora* methods of sporulation (FIGURE 10) as noted in its constituent species, (3) it covers without strain the different varieties of *Pedrosoi*, (4) it leaves out fungi that are exclusively *Hormodendrum*, *Acrotheca*, or *Phialophora* in type (FIGURE 10) (5) it recognizes the existence of a characteristic *Fonsecaea* method of sporulation which is present in all the known strains of the group (FIGURE 8), reaching its highest degree of development in *Fonsecaea Pedrosoi typicus* (FIGURES 8a and 8b) (6) as a generic name, it is neither misleading nor confusing and [illegible]ally (7) *Fonsecaea* has already become extensively popular as noted in a [rev]iew of recent publications on chromoblastomycosis

[I]n view of the fact that the species *Pedrosoi* is an imperfect fungus possess

ing exceptional morphologic characters which link it closely to different form genera it seems probable that differences of opinion regarding its generic position will continue to exist in the future However, we believe that with the broad con
Pedrosoi into
more effective
isolates of this specific group It should be understood that this taxonomic problem will not be definitely solved until the perfect phase of *Fonsecaea Pedrosoi* becomes known

Phialophora verrucosa Medlar, 1915[17] *Synonymy* *Cadophora americana* (Melin and Nannfeldt[59])

Geographic Distribution *Phialophora verrucosa* has been encountered in only six chromoblastomycotic infections The majority of these infections occurred in continental United States, only two cases occurring abroad one in Uruguay[6] (South America), and one in Algiers[61] (North Africa) Although the small number of *Phialophora* infections does not warrant final conclusions regarding the geographic distribution of the fungus as yet, it is of interest to note that five of the isolations were made in temperate climates while only one the North African strain, corresponds to a tropical climate

Natural Habitat Kress *et al*,[60] who worked with material from the wood

by Conant and Martin[54] Thus *Phialophora verrucosa* has become an additional member of the small group of pathogenic fungi so far discovered in their natural environment

Morphology in Pathologic Tissue In parasitized cutaneous tissue, the fungus is indistinguishable from *Fonsecaea Pedrosoi*

Gross Morphology in Culture Healthy colonies are produced in various laboratory media, with great variations in the rate of growth according to the individual isolate cultures three weeks old in dextrose agar ranging in diameter between 0 5 and 3 5 cm Gross cultural characters are generally more evident between the sixth and eighth weeks of growth (FIGURE 11a) The cultures are roughly conical, measuring 3 5 to 6 cm in diameter on the eighth week, with a summit elevation of 5 to 10 mm The surface is irregular, the border festooned or indented, and the aerial mycelium is profuse short, gray, dark olivaceous or dark brown, forming a velvety or feltlike growth

Microscopic Morphology in Culture The microscopic characters are usually more conspicuous between the fourth and sixth weeks of growth The vegetative hyphae are straight or cylindrical, branching and septate with articles 8 to 25 microns long by 2 to 6 m in diameter, septation often closer, forming oidioid or moniliform , the cell walls are coarse and dark brown, with the cell membra hyaline, the protoplasm is yellowish brown fi el granular, and c tile droplets, the nucleus is not appare preparations ith hematoxylin stain The conidiop er terminal or rs 11b 11c, and 11d)

and unicellular measuring 5 to 12 by 2 to 3 8 microns each conidiophore consisting of three parts the base largest of all containing protoplasm and nucleus a constricted portion, or neck, and a cup-shaped outlet ranging in diameter at the lip from 1 1 to 4 7 microns, usually from 2 to 3 As a rule the neck and cup form the terminal portion of the conidiophore which thus becomes flask shaped otherwise cup and neck arise laterally from fertile hyphal article The conidia are semiendogenous, produced singly and successively by budding through the neck into the cup often numerously over flowing the cup and agglutinating to form spherical spore masses loosely adherent to the cup (FIGURES 11b 11c and 11d) The conidial elements are oval sometimes elongated The surface is smooth the wall thin and hyaline measuring 0 8 to 2 8 by 1 4 to 7 8 microns mostly 2 to 4 by 1 to 2 Chlamydospores resembling sclerotic cells are noted in tissue sparsely produced in artificial cultures





FIGURE 11 *Phialophora verrucosa* culture eight weeks old (a) developed at room temperature on Sabouraud's [illegible] (b, c [illegible]) and terminal phialides, and tendency of conidia to aggregate [illegible] the opening of the conidiophore (b and d)

Biologic Characters and Reactions *Phialophora verrucosa* thrives best on an acid substrate The hydrogen ion concentration of the media used in cultural studies of this fungus ranges between 4 3 and 7 Its optimum temperature has not been precisely determined but the fungus is known to develop well at 37°C and satisfactory cultures may be obtained at room temperature (24° to 33°C) A temperature of 100 C is lethal to the parasite in 15 minutes while a temperature of 50°C is well tolerated for at least an hour The development of mycelium and sporulation in the depth of the substrate especially in Czapek's agar indicates that the organism may grow to a certain extent at reduced oxygen tensions

Medlar[?] found that in smears from cultures the fungus elements would stain well though diffusely with all the routine bacteriologic stains structural details being more evident after staining with dilute methylene blue for 10 minutes or longer followed by thorough washing in water

stated that in fresh preparations of the mycelium stained with hematoxylin practically all the cells revealed the presence of a nucleus and in the case of conidia the nucleus also becomes visible with eosin and methylene blue. Young fungus cells would be Gram positive while the older structures would take the blue irregularly.

According to Medlar[12] *Phialophora verrucosa* would form no pellicle in litmus milk; the milk is not coagulated or peptonized and is gradually made alkaline. No indol is produced in Dunham's peptone solution, the medium becoming dark brown to chocolate brown in old cultures. The fungus is a nonfermenter of sugars.

Conant and Martin[31] demonstrated that the serum of a rabbit artificially immunized against *Phialophora verrucosa* possessed complement fixing antibodies in high titer for this species and in a lower titer for the species *Fonsecaea Pedrosoi*. It was further shown that anti-*Pedrosoi* and anti-*compactum* rabbit sera which produced intensely positive reactions with the respective homologous fungus antigens would also react with *Phialophora verrucosa* in a comparatively lower titer.

Taxonomy. The species *Phialophora verrucosa* has not shown a sexual phase of reproduction in laboratory cultures and should be classed, therefore, among the Fungi Imperfecti. It is remarkable that the semiendogenous spores produced by this species resemble the spermatia noted in certain types of Ascomycetes. However, attempts to induce ascus formation by the pairing of different strains in laboratory cultures have not been successful (N. F. Conant, personal communication to the author).

The production of conidiophores from superficial hyphae at any point on the surface of the thallus places this fungus in the third order of Saccardo's classification, namely, the Hyphomycetales (Moniliales), while the dark color of the cultures sets it among the Dematiaceae. Since the conidia are dark and unicellular, the position of the fungus lies in the first division of this family, with the Phaeosporae. According to the method of conidial formation it falls in the subdivision Chalareae. The species was placed in the new genus *Phialophora* Medlar[1?] at the suggestion of Thaxter due to the agglutination of the conidia into sporangium-like masses at the mouths of the phialides. Finally, the specific name *verrucosa* was selected because the lesions produced by the fungus resemble those of tuberculosis verrucosa cutis.

Fonsecaea compactum (Carrion) Carrion 1940 comb. nov.[15] *Synonymy*: *Hormodendrum compactum* (Carrion 1935[?,2]); *Phialoconidiophora compactum* (Moore and Almeida 1936[43]); *Phialophora compactum* (Binford *et al.* 1948[48]).

Geographic Distribution. Up to the present time there are only two recognized representatives of the species *Fonsecaea compactum*. One of them is the fungus originally discovered in a case of chromoblastomycosis in Puerto Rico;[?,23] the other was isolated recently from the skin lesions of a patient in the state of Tennessee (U. S. A.).*

* This isolate was kindly sent to our laboratory by Dr. Norman F. Conant of Duke University, North Carolina.

Morphology in Pathologic Tissue The morphology of this fungus in pathologic tissue (FIGURES 12a and 12b) is essentially similar to that already described for *Fonsecaea Pedrosoi*

Gross Morphology in Culture Growth of the fungus is slow in all the usual laboratory media On Sabouraud's *milieu d'epreuve* and 4 per cent glucose agar (FIGURES 12c and 12d) the fungus when grown at room temperature produces colonies that are roughly conical in shape measuring about 2 5 cm in diameter, with a summit elevation of approximately 6 mm at the end of the sixth week The center of the cultures forms an irregular mammillary prominence about which the colony slopes down unevenly toward a shallow marginal zone The border is irregular and indented

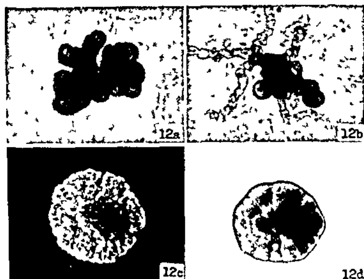


FIGURE 12 *Fonsecaea compactum* (a) aggregate of fungus cells resembling sclerotium in the infected epidermal scales (note internal septation in some of the cells) (b) germination of fungus cells in the infected epidermal scales (c) culture six weeks old developed at room temperature on Sabouraud's *milieu d'epreuve* (d) culture six weeks old developed at room temperature on 4 per cent glucose agar

The aerial hyphae are tufted, forming a plush like or velvety brownish-black growth On Czapek's agar the cultures are poorly developed measuring 10 to 16 mm at the end of the sixth week with the mycelial growth chiefly in the substrate, the center of the colony showing a scant brownish black powdery, aerial growth

Microscopic Morphology in Culture Microscopic characters are usually more conspicuous after the third week of growth Vegetative hyphae are *long coarse (2 5 to 5 2 microns) septate, branching sometimes dichotomously* borders usually irregular cell walls thick and dark, protoplasm olivaceous granular with refractile droplets hyphal fusions frequent, nucleus not apparent in fresh preparations The sporulation is of three dif

stated that, in fresh preparations of the mycelium stained with hematoxylin practically all the cells revealed the presence of a nucleus, and, in the case of conidia, the nucleus also becomes visible with eosin and methylene blue Young fungus cells would be Gram positive, while the older structures would take the blue irregularly

According to Medlar,[12] *Phialophora verrucosa* would form no pellicle in litmus milk, the milk is not coagulated or peptonized and is gradually made alkaline No indol is produced in Dunham's peptone solution, the medium becoming dark brown to chocolate brown in old cultures The fungus is a nonfermenter of sugars

Conant and Martin[51] demonstrated that the serum of a rabbit artificially immunized against *Phialophora verrucosa* possessed complement fixing antibodies in high titer for this species and, in a lower titer, for the species *Fonsecaea Pedrosoi* It was further shown that anti-*Pedrosoi* and anti-*compactum* rabbit sera, which produced intensely positive reactions with the respective homologous fungus antigens, would also react with *Phialophora verrucosa* in a comparatively lower titer

Taxonomy The species *Phialophora verrucosa* has not shown a sexual phase of reproduction in laboratory cultures and should be classed, therefore, among the Fungi Imperfecti It is remarkable that the semiendogenous spores produced by this species resemble the spermatia noted in certain types of Ascomycetes However, attempts to induce ascus formation by the pairing of different strains in laboratory cultures have not been successful (N F Conant, personal communication to the author)

The production of conidiophores from superficial hyphae at any point on the surface of the thallus places this fungus in the third order of Saccardo's classification, namely, the Hyphomycetales (Moniliales), while the dark color of the cultures sets it among the Dematiaceae Since the conidia are dark and unicellular, the position of the fungus lies in the first division of this family, with the Phaeosporae According to the method of conidial formation, it falls in the subdivision Chalareae The species was placed in the new genus *Phialophora* Medlar,[12] at the suggestion of Thaxter, due to the agglutination of the conidia into sporangium like masses at the mouths of the phialides Finally, the specific name *verrucosa* was selected because the lesions produced by the fungus resemble those of tuberculosis verrucosa cutis

Fonsecaea compactum (Carrión) Carrión, 1940, comb nov [13] *Synonymy Hormodendrum compactum* (Carrión, 1935[1,2]), *Phialoconidiophora compactum* (Moore and Almeida, 1936[43]), *Phialophora compactum* (Binford *et al*, 1948[48])

Geographic Distribution Up to the present time there are only two recognized representatives of the species *Fonsecaea compactum* One of them is the fungus originally discovered in a case of chromoblastomycosis in Puerto Rico,[21, 22] the other was isolated recently from the skin lesions of a patient in the state of Tennessee (U S A) *

* This isolate was kindly sent to our laboratory by Dr Norman F Conant of Duke University North Carolina

Morphology in Pathologic Tissue The morphology of this fungus in pathologic tissue (FIGURES 12a and 12b) is essentially similar to that already described for *Fonsecaea Pedrosoi*

Gross Morphology in Culture Growth of the fungus is slow in all the usual laboratory media On Sabouraud's *milieu d epreuve* and 4 per cent glucose agar (FIGURES 12c and 12d), the fungus when grown at room temperature, produces colonies that are roughly conical in shape, measuring about 2 5 cm in diameter with a summit elevation of approximately 6 mm at the end of the sixth week The center of the cultures forms an irregular mammillary prominence about which the colony slopes down unevenly toward a shallow marginal zone The border is irregular and indented

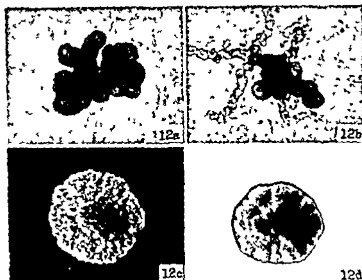


FIGURE 12 *Fonsecaea compactum* (a) aggregate of fungus cells resembling sclerotium in the infected epidermal scales (note internal septation in some of the cells (b) germination of fungus cells in the infected epidermal scales (c culture six weeks old developed at room temperature on Sabouraud s *milieu d epreuve* (d culture six weeks old developed at room temperature on 4 per cent glucose agar

The aerial hyphae are tufted, forming a plush like or velvety brownish-black growth On Czapek s agar the cultures are poorly developed measuring 10 to 16 mm at the end of the sixth week, with the mycelial growth chiefly in the substrate, the center of the colony showing a scant brownish black powdery aerial growth

Microscopic Morphology in Culture Microscopic characters are usually more conspicuous after the third week of growth Vegetative hyphae are long coarse (2 5 to 3 2 microns) septate, branching sometimes dichotomously borders, usually irregular cell walls thick and dark protoplasm olivaceous granular, with refractile droplets hyphal fusions frequent, nucleus not apparent in fresh preparations The sporulation is of three dif

stated that in fresh preparations of the mycelium stained with hematoxylin practically all the cells revealed the presence of a nucleus and in the case of conidia the nucleus also becomes visible with eosin and methylene blue Young fungus cells would be Gram positive while the older structures would take the blue irregularly

According to Medlar[19] *Phialophora verrucosa* would form no pellicle in litmus milk the milk is not coagulated or peptonized and is gradually made alkaline No indol is produced in Dunham s peptone solution the medium becoming dark brown to chocolate brown in old cultures The fungus is a nonfermenter of sugars

Conant and Martin[31] demonstrated that the serum of a rabbit artificially immunized against *Phialophora verrucosa* possessed complement fixing anti bodies in high titer for this species and in a lower titer, for the species *Fonsecaea Pedrosoi* It was further shown that anti *Pedrosoi* and anti *compactum* rabbit sera which produced intensely positive reactions with the respective homologous fungus antigens would also react with *Phialophora verrucosa* in a comparatively lower titer

Taxonomy The species *Phialophora verrucosa* has not shown a sexual phase of reproduction in laboratory cultures and should be classed therefore among the Fungi Imperfecti It is remarkable that the sem endogenous spores produced by this species resemble the spermatia noted in certain types of Ascomycetes However attempts to induce ascus formation by the pairing of different strains in laboratory cultures have not been successful (N F Conant personal communication to the author)

The production of conidiophores from superficial hyphae at any point on the surface of the thallus places this fungus in the third order of Saccardo s

color

are

of

this family with the Phaeosporae According to the method of conidial formation it falls in the subdivision Chalareae The species was placed

due to

uths

ause

icosa

cutis

Fonsecaea compactum (Carrion) Carrion 1940 comb nov[15] *Synonymy* *Hormodendrum compactum* (Carrión 1935[21]) *Phialoconidiophora compactum* (Moore and Almeida 1936[43]) *Phialophora compactum* (Binford *et al* 1948[19])

Geographic Distribution Up to the present time there are only two recognized representatives of the species *Fonsecaea compactum* One of them is the fungus originally discovered in a case of chromoblastomycosis in Puerto Rico [21, 22] the other was isolated recently from the skin lesions of a patient in the state of Tennessee (U S A) *

* This isolate was kindly sent to our laboratory by Dr Norman F Conant of Duke University North Carolina

old on glucose peptone agar developed at 30°C showed ' short chains of spherical oval rod shaped clavate or swollen cells branching was definite though rudimentary dichotomous sometimes trichotomous and would take place at the tip or side of the cell often close to an intercellular septum The

internal septum perpendicular to the longitudinal axis

Large chlamydospores of sclerotic type were present in old cultures Some of these chlamydospores showed internal septation in one or more plains some showed constriction at the line of contact of the cell wall with the internal septum and some produced daughter cells by lateral sprouting

The optimum temperature of the fungus appears to be between 20° and 30°C At 37°C the development was very poor and at 43°C it was inhibited

Candida *like Species* This black *Candida* like fungus was repeatedly isolated by Berger *et al* [35] from the cutaneous lesions of a Canadian patient who had been suffering from papillomatous growths over a period of four teen years Histopathologically the lesions presented the classical picture *of chromoblastomycosis although the fungus was smaller more delicate* and distinctly budding

In the host tissues the fungus appeared somewhat different in two series of biopsies done at different periods In the earlier biopsies the parasitic bodies were very abundant rather small and delicate and had fine mem brane some of them showing distinct budding while others would form

In his description of the fungus as originally isolated Berger reports Colonies on corn meal and hay infusion agar Loeffler s serum and blood agar remain small (3 to 4 mm) and die soon on the two latter media but are larger on glycerolated potato and carrot and on Sabouraud s agar (4 to 7 mm) and still larger on Lowenstein s and Petragnani s media (up to 15 mm) On Sabouraud s agar they are dark brown from the beginning and half-spherical on the other media they are coal black they are always moist of creamy consistence and glossy In potato water (Langeron and Talice) small fluffy colonies rest on the bottom and the liquid contains a great number of single or budding yeast cells there is no veil Cul tures made from a later biopsy (1944) yielded brown to black colonies

of only a scant pseudomycelium at lower pH s pseudomycelial branching filaments extend on the surface and into the underlying medium and bear clusters of pseudoconidial blastospores there are no aerial hyphae In cultures obtained from a later biopsy (1944) the fundamental characters

of filaments forming long branching chains, the acrogenous chains being longer and more branched than the pleurogenous The spores are firmly linked to immediate neighbors in the chain by broad articulations and are olive green the older containing black pigment deposits Some of the spores are spherical (2 to 9 microns in diameter) or oval (2 5 to 14 by 1 4 to 8 microns) but most are irregular in shape (2 to 2.5 by 1.5 to 16 microns) the irregularity resulting from development of knob like or short filiform appendages on the tip or on the side, never on the base of the spore cell These appendages sometimes produce a secondary element and often are separated from each other and from the mother spore by septation

Chlamydospores developed in old cultures sometimes in chains elements double-contoured light brown to brownish black terminal or intercalary

In our fungus collection, two isolates from cases of chromoblastomycosis show essentially the same morphologic characters described by Takahashi for *Torula poikilospora* Careful examination of these isolates in culture however, have revealed though sparsely, the types of sporulation characteristic of *Fonsecaea Pedrosoi* Consequently our two isolates have been classed in the *Pedrosoi* group We have been unable to obtain a culture of Takahashi s organism for comparative studies but are inclined to believe that his isolate like ours is another variety of *Fonsecaea Pedrosoi*

Hormodendrum Species Chromoblastomycosis has been ascribed to authentic *Hormodendrum* species by Simson *et al* [illegible] and O Daly [illegible] respectively in South Africa and Venezuela From the descriptions given by Simson and O Daly as well as from preliminary studies of these fungi carried on in our laboratories it is quite evident that the two isolates are very similar and that they are capable of sporulating by only one method namely the *Hormodendrum*

Hormiscium dermatitidis This fungus was originally isolated from the lesions of a patient with chromoblastomycosis in Japan and the fungus was

cium dermatitidis The histopathology of the nodule was similar to that of other lesions naturally developed on the patient s skin Experimental infections were also produced on mice guinea pigs and rabbits the inoculum consisting of a suspension of the fungus grown in artificial cultures

The morphology of the parasite in pathologic tissue was essentially similar to that already described for other fungi causing chromoblastomycosis Cultures six weeks old developed at room temperature on 4 per cent maltose agar measured 8 mm in diameter and were hemispherical in shape They were coal black in color with the surface finely granular and wrinkled and the substrate stained by black diffusible pigment On 4 per cent grape sugar agar on mulberry and on potato plugs the growth was essentially similar to that on maltose agar On peptone agar the growth was poor and in glucose and maltose peptone water loose floccular colonies developed at the bottom of the tube

The microscopic characters were relatively simple and constant in many laboratory media employed for the study of the fungus Cultures four days

12 MEDLAR E M 1915 A cutaneous infection caused by a new fungus *Phialophora*

17 MEDLAR E M 1915 A new fungus *Phialophora verrucosa* pathogenic for man

18

19

20

21

22

23

2 Chromoblastomy

2 cas de chromomy Ann de dermat

26 1912

27

28

196

29 TAKAHASHI Y 1937 Zur chromoblastomykose (II Mitteilung Uber chromoblastomykose hervorgerufen durch *Hormodendron japonicum* n sp Jap J Dermat & Urol 41 53

30 KEENEY E L L AJELLO & E LANKFORD 1944 Studies on common pathogenic fungi and on *Actinomyces bovis* in vitro effect of sulfonamides Bull Johns Hopkins Hosp 75 393

31 MARTIN D S R D BAKER & N F CONANT 1936 A case of verrucous dermatitis caused by *Hormodendrum Pedrosoi* (chromoblastomycosis) in North Carolina Am J Trop Med 16 593

32 SIMSON F W C HARINGTON & J BARNETSON 1943 Chromoblastomycosis a report of six cases J Path & Bact 55 191

33 O DALY J A 1943 La cromoblastomicosis en Venezuela Rev san y asis social 8 655

34 KANO K 1937 Uber die chromoblastomykose durch einen noch nicht als pathogen beschriebenen pilz *Hormiscium dermatitidis* n sp Arch f Dermat u Syph 176 282

35 BERGER L M BEAUDRY & E GAUMOND 1945 Chromoblastomycosis due to a

36

37

38

39 L

40 M

are still yeast-like, but the cells are larger and thicker, often double walled outlines often irregular, and many elements show internal septations in different planes The resulting multicellular structures become very complicated, and are made up of intricately intertwined, irregularly curved and anastomosing, rather thick threads of clustered elements which grow in all directions " These ' cells may contain one or more round lipoid inclusions ' They "may be considered" as "sclerotic cells and are obviously a variant of the initially yeast-like pseudomycelial fungus, and

In hanging drop and slide cultures, many budding cells may be seen which remain attached to the mother cell, and form, by successive buddings, moniliform strands of various lengths, the latter become pseudomycelial through elongation of the individual elements Apical blastospores appear and give in turn rise to pseudomycelial threads, but the branches are few and the resulting arborization is, therefore, rather simple Some blastospores show intensive budding of many small conidia like blastospores, which form soon more or less large clusters of easily detached cells There is no trace whatsoever of conidiophores or of any other specialized apparatus of sporulation, although many colonies were kept alive for over two years "

Berger's organism grows equally well at room and incubator temperatures, it does not grow in the depth of agar stabs At pH 7, its morphology resembles that of the yeast, and at lower pH's, the cultures become filamentous

The fungus does not liquefy gelatin and has no action on milk It acidifies agar media containing glucose, fructose, mannose, galactose, cellobiose and xylose respectively but there is no gas formation in the corresponding liquid sugar media

Bibliography

1 CARRIÓN A L & M SILVA 1947 Chromoblastomycosis and its etiologic fungi Annales Cryptogamici et Phytopathologici 6 20 Chronica Botanica Co Waltham Mass
2 TERRA F M TORRES, O DA FONSECA & A E AREA LEAO 1922 Novo typo de dermatite verrucosa mycose por Acrotheca com associacao de leishmaniosa Brasil med 36 363
3 PEDROSO A cited by O DA FONSECA & A E AREA LEAO 1930 As chromoblastomycoses Rev Med cir do Brasil 38 197
4 RUDOLPH M 1914 Über die brasilianische figueira " Arch f Schiffs u Tropen Hyg 18 498
5 PEDROSO A & J M GOMES 1920 Sobre quatro casos de dermatite verrucosa produzida pela *Phialophora verrucosa* Ann paulist de med e cir 11 53
6 GOMES J M 1921 Dermatite verrucosa Um novo caso Bol Soc de med e cir de Sao Paulo 4 20
7 MOORE M & F P DE A [illegible] 1935 [illegible] s (chromo- [illegible] d South
8 [illegible] om cosi
9 [illegible] ervación
10 [illegible] new and [illegible] Arch
11 [illegible] edroso e Carrión (cromoblastomycose ou cromomicose) Estado atual da questao Hospital Rio de Janeiro 23 577, 743

HISTOPLASMOSIS AND PULMONARY CALCIFICATION*

By Amos Christie

Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee

Over the years, the pediatrician and the radiologist have come to recognize that there are no pathognomonic changes of primary tuberculous infection in the chest roentgenogram. It can be stated unequivocally, however, that changes, when interpreted in view of the tuberculin test, size, extent and location of the shadows represent our best and most practical means for an accurate diagnosis of primary pulmonary childhood tuberculosis. Without these two diagnostic procedures the pediatrician would be helpless in the differentiation of childhood tuberculosis from other conditions causing infiltration, exudation, and calcification. This is probably why he has been on the defensive in recent years when it became apparent in everyday practice and particularly from mass Roentgen surveys that many children were shown to have massive and multiple calcification usually attributed to tuberculosis, though a considerable number of these were negative to large doses of old tuberculin.

The problem can be stated in another way. It has been considered generally by our colleagues in pathology that calcification in the lungs, the thoracic lymphatic channels and the mediastinal gland structures is due almost invariably to tuberculosis. At the same time, most pediatricians and immunologists believe that tuberculin sensitivity is a relatively stable phenomenon. One of these views must be in error. This can be seen by the fact that in the past twenty years there have appeared in the literature at least twelve reports which show a consistent discrepancy between the tuberculin tests and the prevalence of pulmonary calcification. In these papers summarized in our earlier reports,[1] 27 to 83 per cent of young individuals were found to have pulmonary calcification, a phenomenon generally accepted as indicative of tuberculosis. Yet in each study, one half or less of the individuals were found to have positive tuberculin tests. These reports with few exceptions, were the outcome of studies of young people living in states bordering the Mississippi River and the states of the Western Appalachian slope.

While anergy or loss of sensitivity to tuberculin from complete healing of the tuberculous process may be an acceptable explanation in some cases, we do not believe that this can explain the marked prevalence of pulmonary calcification in this area. We were challenged to investigate other possible causes for these findings.

Theory Behind Histoplasmosis as a Cause of Calcification. Histoplasmosis in a previously unrecognized benign form was not too remote as a possible explanation for some of this calcification. This infectious disease caused by the fungus *Histoplasma capsulatum* occurs in all age groups and, from

* The author is indebted to Doctor Ernest Goodpasture, Professor of Pathology, Vanderbilt University School of Medicine, for permission to include the autopsy findings herein reported and to Dr. James Dawson, who selected the sections and assisted in making the photomicrographs. This also gives me an opportunity to express publicly my thanks to my associate, Doctor J. Cyril Peterson, for his loyal assistance and cooperation throughout all of these studies.

all reports was uniformly fatal This in itself struck us as being unusual. It can be of acute or chronic nature No benign forms had been described. While it is of world wide distribution, more than half of the reported cases have occurred in states which correspond to the area of benign calcification in nontuberculin reactors just discussed It is due to a fungus which occurs in tissues in the yeast forms and ordinarily grows on culture media in the mycelial form Demonstration of the fungus etiology of the disease was first obtained by the epochal cultural and transmission experiments of DeMonbreum[2] at Vanderbilt University Medical School The organism produces lesions which simulate many common diseases It produces ulceration of the mucosa and miliary lesions with caseous necrosis and granulomata which closely resemble tuberculosis This variety of clinical manifestations makes it seem quite possible that benign forms may exist and that the clinical picture might be one such as described in coccidioidomycosis with its primary complex going on to calcification much as in tuberculosis

It had been previously shown by Cox and Smith[3] in 1939 and later by Aronson[4] in 1942 and it is generally recognized in the West that coccidioidomycosis produces a primary complex which goes on to produce calcification quite indistinguishable from that laid down by tuberculosis Prior to these descriptions of benign form, *Coccidioides granuloma* was in somewhat the same state as histoplasmosis It was almost uniformly fatal Smith[5] in 1943 postulated that the area of pulmonary calcification in nontuberculin reactors was also the endemic area of histoplasmosis Infection with *H capsulatum* a fungus closely related biologically to *Coccidioides immitis* might result in previously unrecognized benign forms of infection and the development of the calcifications described These were the reasons behind our suspecting a benign form of histoplasmosis as a cause of our unexplained calcifications

Preliminary Clinical and Pathological Evidence to Support This Thesis In January 1944 a five month old infant, suspected of having leukemia was brought to Vanderbilt University Hospital Subsequent studies proved that he had a miliary histoplasmosis His blood cultures yielded as high as 3 000 fungus colonies per milliliter This child and his parents reacted to histoplasmin an extract of a culture of *Histoplasma capsulatum* which we had prepared

Following this incident we began to test all the children admitted to the pediatric ward with the same lot of histoplasmin and with coccidioidin After approximately 125 children had been tested with both antigens analysis revealed that 23 per cent of the children reacted to histoplasmin while only a few reacted but equivocally to coccidioidin Though there were several very young children who had positive reactions we were unable in any of them to obtain a history of an illness which might have caused the development of sensitivity

Members of the pediatric house staff and medical student body who had lived all their lives in Kentucky, Tennessee and Missouri reacted strongly to the antigen while conversely other members who had recently arrived from California Maryland and New York did not react

As a result of these preliminary skin tests with histoplasmin it seemed apparent (1) that the response to the skin test was the result either of previous infection with *H capsulatum* or of infection with some other fungus having a common or closely related antigen (2) that infection with some other unknown fungus was much more common than could be explained on the basis of any clinically known fungus infection including the dermatomycoses, (3) that infection must at times be almost symptomless

The Infection Histoplasmosis The literature has been filled with case reports of clinically recognizable histoplasmosis starting with the cases of Darling[6] in 1906 and culminating with the reviews of Meleney[7] and of Parsons and Zarafonetis[8] and new cases are being recognized continually Certainly increasing knowledge of the disease has resulted in increasing diagnoses to the point where it is no longer an extremely rare disease While less than a hundred cases have been reported it is safe to say that more are being recognized than are reported

The clinical manifestations of the severe infections are protean These may be epitomized by saying that there is irregular low grade fever hepatomegaly, and splenomegaly with anemia and leucopenia There may be general glandular enlargement and frequently there are symptoms of pulmonary infection pleural pain cough and expectoration Many patients have had symptoms referable to ulcerative lesions of the gastrointestinal tract including the mouth and oropharynx and genitalia Destructive bone lesions occur The duration of the disease in these patients is from a few weeks to as long as 15 to 20 years, and in the great majority the infection has been considered ultimately fatal The diagnosis in these cases can usually be established by adequate cultural techniques and/or the examination of biopsy material lymph nodes and sternal marrow

It is perhaps significant that 18 cases of proven histoplasmosis have been recognized at Vanderbilt Hospital (as of January 1948) and that tissues from a half dozen other cases culled from a group of nonspecific granulomatous lesions and from tuberculin negative cases with morphological diagnoses of hilar tuberculosis are highly suspicious While these cases are of great academic interest they are from our viewpoint much less important than those patients who have minimal nonfatal almost nonsymptomatic infections

Since the relationship of pulmonary calcifications to histoplasmin sensitivity was first pointed out we and others have been engaged in a search for minimal infections In this problem one is faced with the same difficulty that would be encountered after 65 years of extensive investigations in attempting to prove bacteriologically the existence of minimal primary tuberculosis In fact we have found that an additional number of technical difficulties arise which make the solution of the problem a tedious labor

There follows a series of cases which we believe represent the benign or minimal form of the disease

Case 1 A well developed and nourished ten month old infant with bilateral subdural hematomata died from the hyperpyrexia following an attempt at excision of the membrane In the lower lobe of each lung

all reports was uniformly fatal This in itself struck us as being u
It can be of acute or chronic nature No benign forms had been des
While it is of world wide distribution, more than half of the report
have occurred in states which correspond to the area of benign cal
in nontuberculin reactors just discussed It is due to a fungus wl
in tissues in the yeast forms and ordinarily grows on culture me
mycelial form Demonstration of the fungus etiology of the d
first obtained by the epochal cultural and transmission exper
DeMonbreum[2] at Vanderbilt University Medical School The
produces lesions which simulate many common diseases It p
ceration of the mucosa and miliary lesions with caseous n
granulomata which closely resemble tuberculosis This variety
manifestations makes it seem quite possible that benign form
and that the clinical picture might be one such as described in
mycosis with its primary complex going on to calcification
tuberculosis

It had been previously shown by Cox and Smith[3] in 1939
Aronson[4] in 1942, and it is generally recognized in the Wes
oidomycosis produces a primary complex which goes o
calcification quite indistinguishable from that laid down b
Prior to these descriptions of benign form, *Coccidioides gra*
somewhat the same state as histoplasmosis It was almost u
Smith [5] in 1943 postulated that the area of pulmonary
nontuberculin reactors was also the endemic area of histo
fection with *H capsulatum*, a fungus closely related biologic
oides immitis, might result in previously unrecognized be
infection and the development of the calcifications des
were the reasons behind our suspecting a benign form of
as a cause of our unexplained calcifications

Preliminary Clinical and Pathological Evidence to Supp
In January 1944 a five month old infant suspected of hav
was brought to Vanderbilt University Hospital Subsequent
that he had a miliary histoplasmosis His blood cultures y
as 3 000 fungus colonies per milliliter This child and his pa
to histoplasmin an extract of a culture of *Histoplasma capsu*
we had prepared

Following this incident we began to test all the children ad
pediatric ward with the same lot of histoplasmin and with
After approximately 125 children had been tested with both
analysis revealed that 23 per cent of the children reacted to h
while only a few reacted but equivocally, to coccidioidin Th
were several very young children who had positive reaction
unable in any of them to obtain a history of an illness which
caused the development of sensitivity

Members of the pediatric house staff and medical student bod
lived all their lives in Kentucky Tennessee and Missouri reacte
to the antigen while conversely other members who had recentl
from California Maryland and New York did not react

FIGURE 2 Lung (× 375) Showing a tubercle-like focal granulomatous lesion (Case 1)

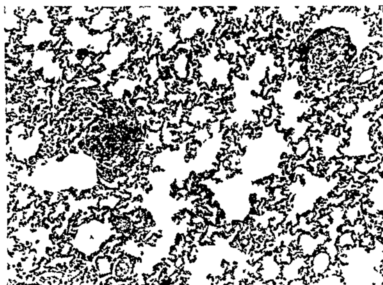


FIGURE 3 Lung (× 120) Showing tubercle-like focal granulomatous lesions (Case 1)

These sections were negative for acid fast organisms They did show a focal or interstitial pneumonitis and the yeast forms of *H capsulatum*



FIGURE 4 Tube le l ke lesion in lung (X 120 f om wh h *H Capsu a um* was cu u ed (Case 2)



F GURE 5 Tuberc e l ke focal granu oma ous le on n lung (X 120 from wh cu tu ed (Case 2

in large mononuclear cells monstrated T' found in the edge of the tu ons seen thro

m either was often of such low virulence or was no longer viable to extent of producing infection because of the action of the tissues ntly my associates[9, 10] at Vanderbilt University School of Medicine ceeded in cultivating the organisms from the lung and lymph node ns of minimal cases such as I have shown here
is our thesis therefore that infection with *H capsulatum* like *Coccidi ›mmitis* or the tubercle bacillus can result in overwhelming infec with dissemination and fatal outcome Depending on the resistance he host the virulence of the organisms or other unknown factors the anisms are rendered nonviable or phagocytized as seen in one of the s shown Giant cell formation around the liberated lipid substances ollowed by caseous necrosis with healing and calcification We have n calcification in a four month-old infant who died of disseminated histo smosis During these later states it will be unlikely that the organisms viable and we should not expect to be able to get positive cultures feel that the focal interstitial or atypical pneumonitis cases of early will be more fruitful for finding the organisms culturally or in tissues s correlates with the age distribution for the development of sensitivity the skin test material which we have shown before[1, 11 12]
Histoplasmin Sensitivity and Pulmonary Calcification In the five years ce the relationship of histoplasmin sensitivity to pulmonary calcification s first shown the aroused interest has resulted in the publication of a nsiderable number of papers on the subject Also considerable effort is been expended in attempts to demonstrate a benign type of infection ith *Histoplasma capsulatum* We will now attempt to review what we ink is significant of this material and to discuss the specificity of the ntigen

General Considerations Preliminary studies with histoplasmin tests by embers of the Department of Pediatrics at Vanderbilt University in 1945 showed that histoplasmin sensitivity was not uncommon and that ich sensitivity was sometimes associated with pulmonary calcifications ot attributable to tuberculosis Palmer[13] studying nurse cadets was ble to show a high degree of correlation between pulmonary calcification d histoplasmin sensitivity He showed that while only 25 per cent of group as a whole reacted to histoplasmin 85 per cent of those with monary calcification reacted to this antigen Our studies[1 11 12] ave likewise shown that 87 per cent of Tennessee residents with calcifica ion react to histoplasmin though such sensitivity is only half that common n those not having calcifications Only 18 8 per cent of those with cal ifications react to tuberculin wh per cent of the group as a whole eact to this antigen

On the other hand, Olsen Bell ›ons[14] in a study of the residents of Loudoun County, Virginia e to find a positive association between a reaction to histoplas rculin and the presence of pul monary calcification, though the rrelation between residence in a tuberculous household and the ulmonary ›tion Waring and Gregg[15] using ou n mad in Charleston South Carolina ly e› of children n with pul

died a postoperative death from congenital biliary atresia We have also found *H capsulatum* in biopsy material of two cases of chronic cervical adenitis These cases will be reported in detail in a subsequent publication now in preparation

While this work is still in a preliminary phase, it gives excellent promise of establishing satisfactory biological proof of the existence of minimal infections Surely the repetition of these findings has reached a proportion where they could not all be early disseminating cases These then, we believe are cases of minimal or benign histoplasmosis proven both histologically and culturally

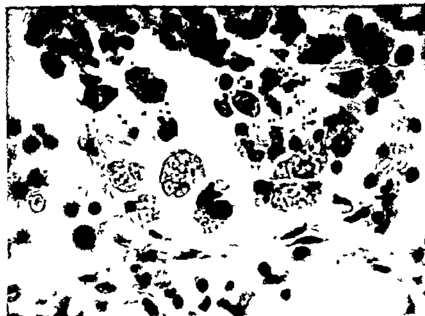


FIGURE 8 Yeast cells in mononuclear phagocyte of lymph node (X 510) (Case 3)

We have been trying to culture the organism from sputum or gastric washings In tissues *H capsulatum* yeast cells are found almost entirely intracellularly and may multiply chiefly if not exclusively within the cells Thus, unless the lesion goes on to caseation and ulceration, one cannot expect the release of organisms and the sputum or gastric washings would be negative This expectation has been confirmed in several ways We have been unable to demonstrate or cultivate the organisms from the sputum in a single case with a fatal miliary infection and have failed in other instances of what seemed to be almost certain infections, to show organisms in the sputum and/or gastric washings

Early attempts to cultivate the organisms from autopsy materials *in vitro* and by passage through experimental animals were unsuccessful—the first because of the invariable contaminants the second because the or

give positive reactions but controls do not react (3) Pilot studies[11] in 281 persons with coccidioidin and in 437 persons with haplosporangin, while yielding many positive reactors to histoplasmin failed to yield a single definite reactor either to coccidioidin or to haplosporangin It would seem that while there might be cross sensitivity in experimental animals these two fungi are not responsible for much of our problem in the area described above We are accumulating evidence that the same can be said for blastomycin (4) The question of *Oidium albicans* has been suggested by Doctor Emmons and others as a possible explanation for some of our sensitivity to histoplasmin The failure to find many young infants sensitive to histoplasmin would make it seem very unlikely that infection with the thrush organisms can be of real significance in relation to histoplasmin sensitivity Oral thrush is a common infection of infants in this section and would if it produced cross sensitivity reactions be likely to yield a high percentage of infantile reactors Furthermore, thrush is not a commonly recognized infection in the older age group where we commonly find histoplasmin sensitivity (5) Age distribution The pattern of age

dying with proven histoplasmosis and we have seen only one other positive reaction in an infant less than a year of age The rapid increase in sensitization in the next few years is certainly not correlated with any particularly developmental achievement It is rather like the development of Schick negativeness in the nonimmunized urban groups a phenomenon no longer generally believed to be due to natural development but generally attributed to immunization resulting from subclinical infections (6) The geographic distribution of histoplasmin sensitivity offers strong support for the supposition that the reaction is a response to an infectious process Geographical variations in the prevalence of pulmonary calcifications have been shown by a number of investigators most strikingly by Long and Stearns [9] They showed that Army inductees from the Western Appalachian slope and the bordering states west of the Mississippi and north of the Ohio River showed a greater prevalence of pulmonary calcifications than those from other areas The studies of Palmer[17] and our studies[1, 12] have shown that histoplasmin sensitivity is much more prevalent in this area of excessive pulmonary calcifications The contrasting study of Waring and Gregg[13] seems of great significance in this respect They found pulmonary calcification in only 3 4 per cent of their school children in an area where the index of histoplasmin sensitivity was low as contrasted with over 40 per cent in the group reported by Olsen Bell and Emmons [14] 55 per cent in our study groups and 12 7 per cent in the group studied by Furculow High and Allen,[21] the latter groups being from areas of high prevalence of histoplasmin sensitivity

We believe that these studies clearly indicate an intimate relationship between pulmonary calcification and histoplasmin sensitivity in the areas where such sensitivity is highly prevalent

Histoplasmin sensitivity prevalence has been shown in the foregoing to

vary greatly with geographical areas We can also add that, in addition to the absence of reactors in Ireland [18] none of several hundred individuals *living in Holland reacted to histoplasmin, there were no clear-cut positive* reactions in more than a hundred persons tested in Curacao, and positive tests are encountered very infrequently in children in New York City, Buffalo and Rochester, New York, in Detroit, in New Orleans, and in San Francisco[22]

It has also been observed in Tennessee that sensitivity to histoplasmin is more prevalent in areas adjacent to streams and other areas of dampness than it is on high dry ridges [23]

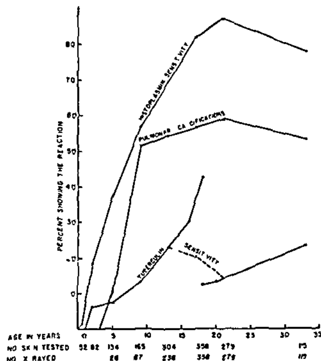


Figure 9 Age relationship in the development of histoplasmin sensitivity pulmonary calcification and tuberculin sensitivity

From these studies it is apparent that the question of histoplasmin sensitivity and its relationship to pulmonary calcifications is one which may be of great importance in one locality and of no importance in another and also that some factors of climate and physical geography must be of great significance in the epidemiology of histoplasmin sensitivity

Our studies[13] show that there is a wide variation in the prevalence of sensitivity from one area to another within the state of Tennessee and in neighboring states. Indeed, the extensive studies reported by Palmer[4] and by Prior and Allen[25] showed that there was a similar variability in other states

Furthermore, the wide variation in sensitivity observed in our studies in students from different states shows that the variation is in relation to a fairly definite geographic focus and is suggestive of an infectious agent existing commonly in an area where climatic factors favor its propagation

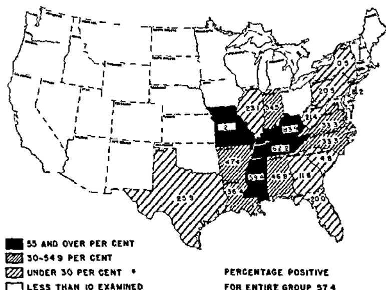
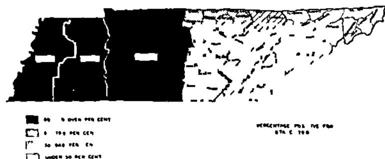


Figure 10 D but on by sta e of res den e of percentage of pe sons exam ned at Vanderb lt Un versity in 194 react ng to h stopla m n



F G RE 11 Percen age of persons exam ned at Vanderb lt Un ve s y and Tennessee Industr al S hool rea t ng to h stoplasm n by county of residen e 1945

T [illegible]

None of these studies prove a direct causal relationship between pulmonary calcifications, histoplasmin sensitivity, and the disease, histoplasmosis The histoplasmin sensitivity may be merely an index of the over-all problem of pulmonary and systemic mycotic infections It may

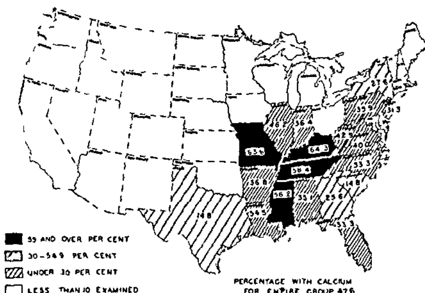


FIGURE 12 Distribution by state of residence of percentage of persons examined at Vanderbilt University in 1945 found to have calcification

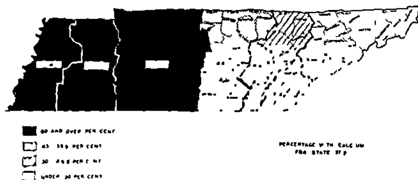


FIGURE 13 Percentage of persons examined at Vanderbilt University and Tennessee Industrial School found to have calcification by county of residence 1945

explain only a segment of our problem of pulmonary calcification in non reactors to tuberculin Further studies of cross-sensitivity among fungus antigens and infections are needed Further study to establish the exact etiologic agent or agents responsible for the histoplasmin sensitivity and pulmonary calcifications is imperative My associates, Doctor Peterson

and Doctor McVickar, and I are engaged in these studies at the present time *

Another approach to the solution of the problem of the minimal infection has been the study of pulmonary roentgenograms in children who have recently developed histoplasmin sensitivity Infants being followed in well baby clinics are given repeated histoplasmin tests and as sensitivity develops roentgenograms are made In a number of these infants we have been able to show pulmonary lesions which were not giving rise to significant symptoms These infants have not been followed long enough to determine whether these lesions go on to calcification In two instances, we have observed hilar lymphadenopathy of a degree sufficient to produce atelectasis and cough in young children who were repeatedly tuberculin [illegible]

histoplasmosis in a benign form

Discussion and Summary These findings seem to have definite significance from the viewpoint of the interpretations of the mass roentgenographic studies now being made and contemplated It seems quite certain that in states bordering the western bank of the Mississippi River and the states of the Western Appalachian slope a large part of the pulmonary calcifications are due to some infection other than tuberculosis It seems that while this relationship is most striking in the area outlined above, it is not confined to this area but has a much wider application

If these studies have no other value, they do serve to reinstitute and re emphasize the necessity of employing either tuberculin tests or the demonstration of tubercle bacilli in establishing the diagnosis of tuberculosis in minimal and noncavitated pulmonary lesions and in the healing lesions with pulmonary calcifications Present-day concepts of the primary complex, or first infection tuberculosis, in its relationships to pulmonary calcification, need re evaluation The same may be true of reinfection tuberculosis

Conclusions (1) The problem of pulmonary calcification in tuberculin negative individuals has been reviewed (2) The available epidemiological evidence and experience with histoplasmin skin tests to support this thesis have been reviewed and evaluated (3) The significant clinical and pathological evidence suggesting that benign forms of histoplasmosis may be responsible for a segment of this previously unexplained calcification is presented The significance of these observations has been stated

Bibliography

1 Christie A & J C Peterson 1945 Am J Pub Health 35 1131
2 DeMonbreun W A 1934 Am J Trop Med 14 93
3 Cox A J & C E Smith 1939 Arch Path 27 717
4 Aronson J D R M Saylor, & E I Parr 1942 Arch Path 34 31
5 Smith C E 1943 Med Clin No Am 27 790
6 Darling S T 1906 J A M A 46 1283

* Supported by a grant from the National Tuberculosis Association

7 Meleney H E 1940 Am J Trop Med 20 603
8 Parsons R J & C J D Zarafonetis 1945 Arch Int Med 75 1
9 Dawson J R Dept of Path Vanderbilt University Hospital Personal communication
10 Womack F Dept of Path Vanderbilt University Hospital Unpublished data
11 Christie A & J C Peterson 1946 J A M A 131 658
12 [illegible] & J C Peterson 1946 [illegible] 29 417

[illegible] Am J Pub Health 31 441
[illegible] Child 73 139
[illegible] I Magnon 1946 J Med Assoc [illegible] 19 [illegible]
17 Alman R W & M Opinsky 1946 Clin Proc Childrens Hospital Washington D C 2 284
18 Emmons C W B J Olsen & W W Ethridge 1945 Pub Health Reports 60 1383
19 Howell A 1947 Pub Health Reports 62 63
20 Long E R & W H Stearns 1945 Radiology 41 144
21 Furcolow M L R H High & M F Allen 1946 U S Pub Health Reports 61 1132
22 Orie N G M Groningen Netherlands A W Pot Curacao N W I S Z Levine New York N Y R McIntosh New York N Y P J Howard Detroit Michigan C E Smith San Francisco California R V Platou New Orleans Louisiana Personal communications
23 Williamson County Tuberculosis Study Unpublished data
24 Palmer C E 1946 Pub Health Reports 61 475
25 Prior J A & M F Allen 1947 Pub Health Reports 62 1608

CRYPTOCOCCOSIS AND BLASTOMYCOSIS

By Rhoda W Benham

Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York, N Y

The two diseases which form the title of this report were described for the first time in 1894 one from Europe, the other from the United States These two with a number of other distinct diseases, did in the past and in some cases still do appear in the literature under the name "blastomycosis" The resulting chaos in terminology has been difficult to overcome It originated with the practice of referring to organisms reproducing by buds or blastospores as "Blastomycetes," and the conditions in which they occurred as "blastomycosis" Since the etiological agent in many different deep-seated fungus infections appears in the tissues or exudate from the lesions in the form of oval or rounded budding cells they were often erroneously described as blastomycosis Cultures proved, however, that a number of different fungi were involved and that a number of distinct diseases had been described under one name Some workers went so far as to suggest that blastomycosis was a disease entity due to a plurality of species Castellani[1] for one stated in 1920 that "Blastomycosis is a term generally applied to affections due to fungi of the genera *Saccharomyces, Cryptococcus Monilia, Oidium*, and *Coccidioides*"

As a result, then, of cultural studies and of a careful consideration of the characteristic appearance of the fungus in the parasitic state, we now have a number of deep-seated fungus infections described under their respective names and the term blastomycosis has become more limited in scope

s

I

I

and has not much significance otherwise

History shows us that the name *Blastomyces* was first given by Constantin and Rolland[4] (1888) to a fungus which they isolated from dung and described as a new species It is a filamentous, branched form with conidia at the ends of short branches These conidia or buds, as these authors called them, in turn produce so-called secondary buds at many points, and these still others, until each fruiting branch transforms itself into a powdery spore mass This fruiting mass is yellow to sulphur color, passing to red orange and sometimes green Only one species is known, *Blastomyces luteus* This is listed as being in the collection at Baarn, but I have not as yet obtained a subculture Just what it is would be anyone's guess, but, in the illustrations, the conidia are not unlike those of some of the gymnoascus forms or of some of the dermatophytes Certainly it is not a yeast like fungus In Clements and Shear's[5] key to Saccardo's classification we find it defined as follows "Hyphae elongated and distinct from conidia Conidia bearing hyphae of two sorts, the upright alone denticulate"

Just where or how the term *Blastomyces* entered the literature to designate those forms reproducing by a process of budding or by blastospores is not quite clear, but Gilchrist and Stokes[6] appear to have been the first to use it in the medical literature, when they gave the name "*Blastomyces dermatitidis*" to the fungus and the name "Blastomycetic dermatitis" to the cutaneous disease which they described The fact that rounded, budding cells were noted in both their case and the European case described by Busse and Buschke led then to their both being called "blastomycosis" We now know, and it has been pointed out many times, that the organisms are quite different The *Blastomyces* of Gilchrist is a hyphomycete, and the other a yeast like fungus The latter and *Candida albicans* are the only proven yeast like pathogens One would think that with the numerous publications on the subject, the fact that these fungi are quite distinct and different from *Blastomyces dermatitidis* would now be very clear However, in a recently published text, 'The Fungi,'[7] which appeared in two volumes, only es, and the final ni fest as cutaneous abscesses is caused by the *closely related* Blastomyces dermatitidis, also known as Gilchristia dermatitidis or Zymonema dermatitidis' This sentence alone would seem to justify repeating these well known facts Evidently, it is necessary to emphasize again that these diseases are distinct and due to entirely different fungi, and that we should no longer use the term blastomycosis in speaking of Busse and Buschke's disease It is due to a yeast like fungus which reproduces by budding, does not form mycelium or ascospores, and which therefore belongs to the group of the cryptococci *The disease which it causes should be called* "cryptococcosis"

There follows a discussion of these diseases in detail, with brief mention of the clinical and pathological aspects and stress laid on the laboratory diagnosis and the specific fungus involved in each case

Cryptococcosis

History The first description of this infection, according to Freeman[8]

differed somewhat from those that followed, as it was one of the rare cases with cutaneous manifestations The patient, a woman, had an abscess of the tibia from which was obtained a thick, gelatinous exudate in which

involvement of the bones, subcutaneous tissues or, in some instances, the viscera have since been described under the names yeast infection, saccharomycosis blastomycosis, or torulosis

In 1906 von Hansemann[17] in Germany, reported a yeast infection of the brain with similar gelatinous abscesses but without reference to Busse and Buschke's case In 1916 Stoddard and Cutler[13] described two cases of their own which they concluded were identical with von Hansemann's and with three other examples which they found in the literature They described clearly the clinical and pathological differences between this disease and Gilchrist's blastomycosis and the sharply different characteristics of the causative fungi They gave the name *Torula hystolitica* to this fungus and Torula infection to the disease

Unfortunately Stoddard and Cutler were misled by Buschke's assertion (later retracted) that his yeast produced endospores and concluded that his and similar cases were a different disease which they called true yeast infection They stated that torula infection might affect other organs than the central nervous system but never the skin Cases with subcutaneous or visceral involvement continued to be reported as blastomycosis, or under other names

In 1934 Lodder[14] and the author[5] independently reported studies in which the strain of Busse and Buschke was compared with strains from torula meningitis and found indistinguishable This showed that whereas one name blastomycosis had been used for several distinct diseases one disease cryptococcosis had been described under several names

Other reviews and monographs have been published and there is now a vast literature on the subject but time will not permit a discussion of these Attention should be called however to a book which appeared in 1946 by the Australian workers Cox and Tolhurst[18] They list a total of about 120 cases including 33 from Australia This book covers every phase of the disease in a most complete and thorough manner and is invaluable to anyone interested in the subject These authors obtained cultures from 10 cases and state that they believe this to be the greatest number isolated in any one laboratory In our laboratory at the College of Physicians and Surgeons we have identified 9 strains 1 in 1937 and 8 since 1945 This raises the question whether the increase of the disease is actual or should be attributed to better diagnosis

Symptomatology and Treatment Cryptococcosis European blastomycosis or as more commonly known torula meningitis is a chronic infection most frequently limited to the central nervous system though it may involve other organs the skeletal structure and the skin The symptoms are primarily those of severe headache vomiting and stiff neck with little or no fever These are sometimes accompanied by disturbed vision and mental confusion Diagnosis may then be confused with intracranial tumor or tuberculous meningitis and may only be confirmed with certainty by demonstrating the presence of the organism either directly or on culture The respiratory system may be involved but this is uncommon and infection of skin and bones is rare There is a striking absence of inflammatory response in lesions of the brain and meninges In skin or bone abscesses a thick mucoid blood streaked pus forms which appears upon examination to be almost a pure culture of the organism but some granulation tissue

develops about the abscess, and pseudotubercles are described The central nervous system cases are almost invariably fatal No form of treatment has proved successful Spontaneous remissions occur, which make it difficult to evaluate treatment However, Marshall and Teed[17] report a cure with sulfadiazine, and Keeny, Ajello, and Lankford[18] (1944) found that sulfadiazine was effective *in vitro*, whereas sulfathiazole and sulfamerazine were not *Sulfadiazine was ineffective with one of our patients*

There are conflicting reports as to sensitivity to penicillin, and it may be that there are strain variations Streptothricin is reported as active *in vitro*, more so than streptomycin Protoanemonin was shown by Holden *et al*[19] to inhibit the cryptococcus in a dilution of 1-80,000 When injected into a bone abscess in one of our cases, this antibiotic caused the cavity to become sterile after 4-5 weeks

Further search of new antibiotics with which to treat this disease is urgently needed

Diagnosis Serology is of no value in establishing a diagnosis, and the skin test has not been sufficiently studied as yet to be considered reliable, although it has been reported positive in a few cases Spinal fluid findings may be of some value, as it has usually a low chloride and sugar content and is under increased pressure But, as emphasized many times, finding *Cryptococcus neoformans* either directly or in culture is the only certain means of diagnosis It has been demonstrated in, or isolated from spinal fluid, pus, sputum, urine, and blood, the last two having been reported as a source of culture in only very few instances

The material for examination is placed on a slide with a drop of India ink *In such a preparation, the organism appears as an oval or spherical* budding cell with a definite wall surrounded by a clear area, or halo, which constitutes the capsule Gram stain of fixed smears will serve also to demonstrate the fungus, the cell being gram positive, the capsule faintly pink or unstained The fungus may be grown out from the material referred to on the usual Sabouraud's medium or blood agar in 4-5 days at 37° C or in 7-8 days at room temperature

The cryptococci may be defined, as just stated, as yeast-like fungi of oval, globoid, or spherical shape, reproducing by a process of budding They do not form spores or produce true mycelium

Etiological Agent —Cryptococcus neoformans

Nomenclature As the name *Torula* is still commonly used in the medical literature for this fungus, a brief resume of the terminology should be given

The generic name *Cryptococcus* was first given by Kutzing[23] to an organism which he found on moist window panes and which he classified among the algae His herbarium specimen is extant in sufficiently good condition to prove that his fungus multiplied by budding Vuillemin[21] adopted the term for pathogenic yeast like fungi which did not develop ascospores, and gave the name *Cryptococcus hominis* to the Busse Buschke organism There seems as yet no cause to abandon its use

The name *Torula* was first used by Persoon[22] to designate one of the Dematiaceae, a form with short, dark hyphae and chains of globose conidia

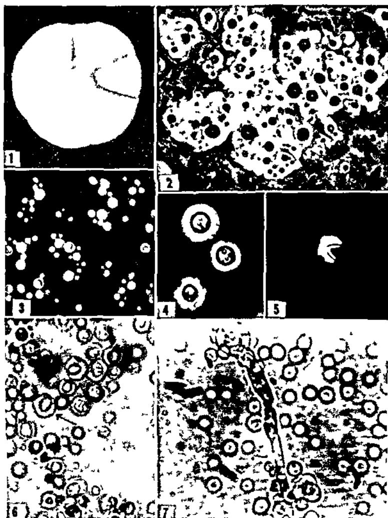


PLATE I. *Cryptococcus neoformans*

FIGURE 1 Colony on dextrose agar 1 month
FIGURE 2 Budding cells in rat tissue—showing capsule (× 400)
FIGURE 3 India ink mount of culture showing spherical budding cells surrounded by narrow capsule. × 160
FIGURE 4 Encapsulated cells from peritoneal cavity of mouse Indian ink preparation (× 400)
FIGURE 5 Empty shell like structure oval body having escaped
FIGURE 6 Culture mount from cornmeal agar showing thick walled or capsulated irregularly shaped cells containing oval bod
FIGURE 7 Culture mount of *C neoformans* showing pseudomycelium

Turpin[23] used the same name for nonsporulating yeast like forms, and both Pasteur[24] and Hansen[25] followed this practice Berlese[26] attempted to correct the error by substituting the name 'Torulopsis" for *Torula*, but although this name was accepted by Saccardo it never came into general use Frothingham[27] and Stoddard and Cutler[13] adopted *Torula* as the generic name for the pathogenic asporogenous yeasts which they studied On the other hand, Guilliermond[28] applied the name *Cryptococcus* to the pathogenic types, and *Torula* to those which were nonpathogenic Lodder[29] (1938) made a plea for the return to 'Torulopsis" as a genus for the asporogenous yeast, and gave as a type species *Torulopsis pulcherrima* Since then, she, with Diddens[30] has shown that *Torulopsis pulcherrima* is a mycelia forming yeast like fungus, and belongs in reality to the genus *Candida* The type species then must be removed from the genus and the generic name is no longer valid We find, therefore, that *Cryptococcus* in respect to both priority and usage, still is the preferred name

As to the specific name, Sanfelice[31] isolated from a fruit a fungus which Lodder many years later found identical to that of Busse and Buschke He named his fungus *Saccharomyces neoformans*, which gives '*neoformans*' priority, and our fungus becomes *Cryptococcus neoformans*—with the *Cryptococcus hominis* and *Torula histolytica* falling into synonymy

A further change may prove necessary, since ascospores have been described in cultures of this fungus (Todd and Herman,[32] Redaelli and Ciferri,[33] and Goetz[34]), and on this basis the organism has been placed in the genus *Debaryomyces* This work, however, needs further verification

pendent of the capsule This also varies in diameter, in some instances being 2 3 times the diameter of the cells, whereas in daughter cells or small cells only a narrow capsule is observed The cells have a distinct wall and contain from few to many granules The contents of the young cells appear to be quite homogenous

In pathological tissues, a variety of forms may be seen Commonly, in the brain, numerous cysts are formed which contain large numbers of small organisms some round, some convex These small organisms do not appear to have a capsule immediately about the cells as do the larger forms In other lesions larger cells are seen which appear to lie in space but in reality are lying in gelatinous material Immediately surrounding these cells which may reach 20 μ in diameter, there is often a dense layer of deeply staining capsular material This material stains red with eosin Sometimes strands of stained material seem to run out from the cell through the capsule giving the cell a spiny appearance These have been noted especially in material from experimental animals and have been regarded as due to fixation Some cells become very large and the wall thickens and eventually ruptures thus destroying the cell Calcified forms have also been noted in animal tissues

Characteristics In Culture *Cryptococcus neoformans* grows readily on

Sabouraud s honey or glucose agar producing at first a creamy tan staphylococcuslike colony which increases in size becomes heaped up with a smooth glossy surface and mucoid consistency or a more dull and dry surface The color varies from light cream to dark tan brown or a deep orange color in some strains Some strains show a tendency to vary producing drier and more pigmented sectors

The capsule forms in culture but shows great variation and is usually not as wide as in tissues Usually it seems wider when the organism is grown on blood agar at 37° C

The cells vary greatly in size and form In young cultures spherical budding cells are seen those of a diameter of 5 6 μ usually with a narrow capsule The bud has a smaller capsule than the parent cell A few large cells will appear and these have wide capsules As the culture ages a characteristic structure appears which has been interpreted by some as an ascus by others as a modified capsule

It appears as though the capsular substance immediately around the cell becomes condensed and merges with the wall or that the wall itself expands and becomes thickened so that a shell forms about a single large granule or mass of smaller granules This globular mass or spore like structure lies at one end of the shell where the wall appears thinner and a somewhat triangular structure results The spore like mass may shed this husk or shell when it ruptures at the thinner portion The spore emerges and buds It may bud while still in the shell Empty shells may be seen throughout the culture This phenomenon whether it be a modified capsule as suggested by Emmons[31] or an ascus as stated by Todd and Herman[32] and others needs further investigation and cytological studies to be thoroughly understood If an ascus it is certainly different from anything noted in other yeasts Occasionally elongated cells or chains of 2 or 3 such cells forming a pseudomycelium are noted both in culture and in animal tissues but nothing in the nature of a true mycelium is seen

Biological The fermentative power is weak There is no gas formation in any sugar and acid only in glucose Strains are reported to liquefy gelatin with n 6 weeks and litmus milk is turned alkaline Little is known about the nature of the capsule Recently Mager and Aschner[33] have demonstrated the presence of 2 polysaccharides One of them turns blue with iodine and is thought to be amylose They state that this reaction may be an aid in the identification of the organisms as this production of extracellular starch seems to be specific for the group of nonfermenting capsulated yeasts to which *C neoformans* belongs

A few studies have been made as to the resistance to heat Cox and Tolhurst[8] report that exposure to 60° for five minutes kills the organism Croyse de Groat and Wahlin[37] report death at 52° for forty two minutes and 60° for five minutes At 42° C many of our strains survived 30–50 hours incubation One which appeared less virulent died out after 5 hours at 42°

Serological The production of antisera in rabbits is difficult I have

been able to produce sera with a titer of 1-160 by treating the cells with acid to remove the capsule before immunizing, and then giving weekly injections for 10 or 12 weeks[15]

A number of strains from various sources, including that of Busse and Buschke, were found to be antigenically identical The tests, however, did not serve to separate the *C hominis* strains from those labelled *T histolytica* One pathogenic strain, the so called *Saccharomyces tumefaciens* of Curtis, was found to be different These reactions did not definitely separate the pathogens from the non pathogens, as several of the latter reacted well with *C neoformans* serum and only feebly with the serum prepared against nonpathogenic strains For instance, a nonpathogenic strain isolated from the feces of a healthy individual proved to be similar antigenically It completely absorbed agglutinins from 3 pathogenic sera, but the reverse did not hold true The pathogenic strains did not react strongly with the nonpathogenic serum

Cox and Tolhurst[16] report sera with a titer of 1-128 by giving 9 injections at intervals of 5 days of a formalized suspension Others have reported negative results in their attempts to immunize rabbits

Further studies are being made with our recently isolated strains in an attempt to produce sera with higher titer and thus render more adequate this method which promises to have value in the understanding of these fungi Complement fixation tests have so far been reported negative, both with the patient's serum and with serum from immunized rabbits

Pathogenicity and Virulence The organism is pathogenic for the usual laboratory animals, with rats and mice the most susceptible, rabbits and guinea pigs less so Virulence does not seem to change on artificial media The strain purported to be the original of Busse and Buschke is still pathogenic for animals The virulence does seem to vary with different strains The animals likewise show individual difference Of any inoculated group, some will show more resistance than others and survive longer, but according to Cox and Tolhurst,[16] no inoculated mouse escapes the infection Lesions in the brain, and other tissues similar to those seen in the human, are encountered

Epidemiology The way in which infection takes place is not clear The disease occurs sporadically in both man and animals and is met with in all parts of the world It does not spread from person to person Organisms similar, except in virulence, for laboratory animals, and in the ability to grow at 37°, have been isolated from the skin and intestinal tract of normal individuals The *Saccharomyces* isolated from fruit by Sanfelice appears identical With these exceptions, *C neoformans* has not been recovered except from lesions Some mutations may account for the pathogenicity The acquiring of a capsule may serve to protect the organisms, which then find the tissues a favorable environment for growth

There is much need for a careful study and comparisons of case histories in the hope that light will be thrown upon the epidemiology of this dreaded disease

Blastomycosis

Gilchr...

. . . astomycosis
. . . and affect other viscera Central
n . . . ous system lesions may follow a preliminary infection of the skin or may arise from some hidden focus, probably the lungs The visceral forms are frequently fatal, and the central nervous system cases invariably so

History The first description of the disease is that of . . . ist[24] and Gilchrist and Stokes[4] In the . . . with a . . . s, round . . . From . . . Gilchrist ob- . . . mycelium This was at first called . . . but later, on the supposition that it was the same as the Busse Buschke organism, it was named *Blastomyces dermatitidis* Other cases were reported and a good description of the disease given by Montgomery and Ormsby[48] in 1908 The infection seems to be limited to North Am . . . a, with cases reported from at least 28 . . . cago . . . one in . . .

. . . many the fungus . . . *. . . myces dermatitides*

Symptomatology The cutaneous form of the disease apparently results from surface infection and the first les . . . s pustules They may . . . the neighborhood, new . . . a plaque with a raised . . . opes down to normal skin The central portion becomes covered with crusts, and miliary abscesses are . . . the margin Larger les . . . areas of the trunk in . . . painless Fever or sub . . .

The systemic variety may, as has been said, follow primary infection of the skin, although, in 50 per cent of the cases, the first symptoms are referable to the respiratory tract Subcutaneous nodules form and, as the disease progresses, break down and ulcerate thus resembling the cutaneous nodules of cryptococcosis Granulomatous nodules may . . . in the lungs, larynx, bones liver k . . .

in a dilution of 1 500,000 (Sanderson and Smith,[41]) but is of little therapeutic value Noojin and Callaway[46] tested seven sulfonamides against *B dermatitidis in vitro* and found that sulfonamilamide and sulfadiazine were the most effective Concentrations necessary to effect the fungus would, however, be too high for clinical use, except perhaps as applied locally to cutaneous lesions Foster and Woodruff[13] report that *Blastomyces dermatitidis* is unaffected by penicillin, even in doses as high as 30 oxford units per cc, but streptothricin inhibits the fungus in concentrations com

skin lesions and used to irrigate the osteomyelitic infection In addition it was mixed

enema Trea

as cured in

causes and, at autopsy, gave no evidence of infection with *Blastomyces*, nor any evidence of harmful effect of the drug The effectiveness of ether has not yet been confirmed by other observers

Diagnosis As with other fungus diseases, the diagnosis can only be made definite by finding the characteristic organism either upon direct examination or in culture Skin tests may be a real aid, as in this case it is a specific reaction similar to a positive tuberculin reaction The reaction appears in 12-23 hours, reaches a maximum in 2-4 days In very allergic patients, the reaction may proceed to a sterile abscess A negative or weak reaction may result in patients in the terminal stages of the disease On the other hand, positive reactions have not been noted in patients without blastomycosis Martin[45] believes that a skin test should always be made before starting treatment with iodides If the reaction is less than 1 cm in diameter, it is safe to administer potassium iodide, but, if 1 cm

injections Surgery also is of value in treatment, and X-ray therapy has proved helpful in clearing cutaneous lesions, provided the hypersensitivity of the patient has been reduced by vaccine therapy

Etiological Agent Blastomyces dermatitidis (Gilchrist and Stokes, 1898)

Nomenclature Many names have appeared in the literature to designate this fungus Some, such as *Oidium*, *Zymonema*, *Acladium*, *Aleurisma*, and *Endomyces*, express the authors' belief as to the botanical classification of the organism Others were given because the authors failed to recognize the identity of their strain with the species described by Gilchrist Ota,[48] as a result of his studies, concluded that a strain isolated by Gammel, and named *Glenospora gammeli* by Pollacci and Nannizzi,[47] and two strains of *Blastomyces dermatitidis* obtained from Weidman were identical, but added to the confusion by naming the organism *Acladium gammeli*, and later with Kawatsure[49] renaming it *Aleurisma tulanense* Castellani[3] had pro

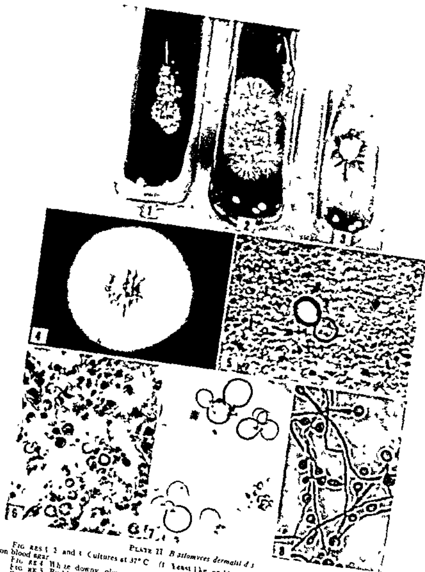


PLATE 11 *Blastomyces dermatitidis*

FIGURES 1, 2 and 3 Cultures at 37° C (1 Yeast like on blood agar; 2 and 3 Intermediate stages on blood agar)
FIGURE 4 White downy colony on Sabouraud's dextrose at room temperature
FIGURE 5 Budding cells in pus direct from lesion (× 400)
FIGURE 6 Budding cells in lung of dog (× 400)
FIGURE 7 Culture at 37 C on blood agar showing yeast like cells (× 400)
FIGURE 8 Culture at 37 C showing mycelium and conidia (× 40)

posed the new genus *Blastomycoides* for the fungi causing American Blastomycosis He made the mistake of including *Blastomycoides immitis* in the genus

In 1934, in a comparative study of the fungi of blastomycosis and coccidioidal granuloma,[49] I showed that *Glenospora gammeli* (Pollacci and Nannizzi, 1927), *Blastomycoides* (monosporium) *tulanense* (Castellani 1928), *Endomyces capsulatus* (Newbridge, Dodge, and Ayers, 1929), *Endomyces capsulatus*, variety *Isabellinus* (Moore, 1933), and *Endomyces dermatitidis* (Moore, 1933) were identical with *Blastomyces dermatitidis* Ciferri and Redaelli[50] also showed these strains to be identical and proposed the name *Gilchristis dermatitidis* Conant[8] once more confirmed these authors and compared many of these same strains with several strains of *Blastomyces dermatitidis* (Gilchrist and Stokes, 1898) isolated from cases of blastomycosis at Duke Dodge[51] applied the name *Zymonema* (Buermann and Gougerot, 1909) to this fungus, giving as the type species, *Endomyces dermatitidis* and placing it in the lower ascomycetes, following Moore's[52] report of asci in this strain The development of asci has not been confirmed, and there seems as yet no valid reason for replacing the name used by Gilchrist, *Blastomyces dermatitidis*

Characteristics In Tissue The fungus appears in the patient's lesion or in those of inoculated animals as round or oval cells usually 8 10 μ in diameter, though often as large as 20 μ They usually produce one bud at a time and the cells are highly granular In sections, the wall is sharply differentiated from the contents, which are somewhat shrunken by fixation, thus pulling away from the wall and leaving a space This, I believe has led to the wall s being referred to as a capsule There is, however, no capsular substance comparable to that of *Cryptococcus neoformans* In tissue, the organisms may be found in large masses but more often mingled with leucocytes in an abscess, scattered through the granulation tissue or phagocytes by giant cells

Characteristics In Culture This fungus assumes a variety of forms, depending on the medium and incubation temperature It grows well on Sabouraud s media and is isolated most easily from the pus from miliary abscesses It takes from 10 days to 2 weeks to appear If the first cultures are incubated at 37° C and on blood agar, a brownish, waxy to smooth growth occurs which Henrici[53] referred to as the mealy" type In this type of growth, the fungus maintains the yeast morphology with only occasional elongated forms, indicating a transitional stage Some cultures become covered with tufts or spiny elevations which resemble coraemia This is Henrici's "prickly" type As soon as the cultures are brought to room temperature, however, the yeast like growth is lost, and an extensive, branching mycelium forms On Sabouraud's maltose or dextrose medium, the fungus grows, slowly forming a downy to fluffy colony, at first white but later deep cream or tan In giant colonies, the central zones may show radial folds and often concentric rings The colony often resembles the *Trichophytons* Ricketts[54] recognized these three types of growth

In these downy colonies, rather characteristic conidia, first described by Gilchrist, are borne on the sides of the hyphae or on the tips of short lateral branches They appear first as knoblike projections, which gradually enlarge At first, the wall is thin and the contents are more or less homogeneous Later, the walls thicken and the contents become more granular, giving somewhat the appearance of chlamydospores These cells may become free and appear to bud, resembling the cells observed in tissue These conidia are found regularly in the downy or fluffy colonies All transitional forms, from yeast to fluffy, may be seen

Biological As reported by Levine and Ordal,[54] *B dermatitidis* does not seem to require accessory growth factors The growth on a medium of salts glucose, and ammonium sulfate is never equal to that on a more complete peptone glucose medium This would indicate a stimulating action of amino acids On a complete medium, the organism grew well at all pH levels except 5 5 at room temperature, but at 37° no growth occurred at pH 4 5 or below On a deficient medium, the quantity of growth increased as pH concentration decreased Also, in general, the higher the temperature is, the greater the amount of growth The effect of temperature on the form of growth has already been mentioned The tendency to form mycelium decreases as the hydrogen ion concentration and temperature increases

Serology Sera of patients with extensive lesions will fix complement with suspensions or extracts of *B dermatitidis* No cross reaction with extracts of other fungi occur, and the reactions are always negative in the absence of blastomycosis

In mild infections that is, in patients with the localized cutaneous forms, no fixation takes place According to Martin,[55] a fatal outcome is to be expected in patients with a high antibody titer and a negative or slightly positive skin test Prognosis is best in the hypersensitive patient without complement fixing antibodies in his serum

Pathogenicity and Virulence Pathogenicity, as in the case of the cryptococcus seems to be a fixed character, for, after years on artificial media, this fungus will reproduce the disease in animals Of the laboratory animals, dogs and monkeys seem most susceptible Heavy subcutaneous inoculation results in a local suppurative granuloma, with metastatic lesions in the lungs and other viscera Spring[56] found mice more susceptible than guinea pigs and rabbits In mice typical lesions should develop in 3 weeks with lesions in liver, spleen lungs and lymph nodes Examination of the fresh material from these lesions or from the peritoneal fluid will show the typical parasitic stage Baker[57] attempted to enhance the virulence for mice by repeated transfer, but failed to do so

Epidemiology The source of the infection is unknown Stober[58] observed in rotting wood from the rooms where patients with blastomycosis had lived, fungi which resembled the parasite, but was unable to prove their identity Infection however probably is from some exogenous source The disease has been known to occur spontaneously in animals Foshay and Madden[59] reported the first spontaneous case in a dog Th

was obtained on culture, and the serum from the dog was completely fixed with typical *Blastomyces* strains

Summary

In this report, attention has been called to the fact that the disease described by Busse and Buschke, in Europe in 1894, and those meningeal infections later described by Von Hansemann, Stoddard and Cutler, and others are really one and the same disease, caused by a yeast like fungus *Cryptococcus neoformans* Also, it has been indicated that North American blastomycosis is an entirely different disease, caused by a mycelium producing fungus, *Blastomyces dermatitidis* A detailed consideration of these two fungi has been given and the correct terminology discussed Once more, the suggestion is made that the term *Blastomyces* be discontinued, except as it pertains to Gilchrist's disease, and that *Blastomyces dermatitidis* be made a *nomen conservandum* for the fungus involved Likewise, it is suggested that the name cryptococcosis replace torulosis or torula meningitis for the meningeal cases, and that *Cryptococcus neoformans* also become a *nomen conservandum*

Bibliography

1 CASTELI [illegible] patho [illegible]
2 CASTELI [illegible] plural [illegible]
3 CONANT [illegible] Stoke [illegible]
4 COSTAN [illegible] Myc [illegible]
5 CLEMENTS, F C & C L SHEAR 1931 The Genera of Fungi H W Wilson. New York
6 GILCHRIST T C & W R STOKES 1898 A case of pseudo-lupus vulgaris caused by [illegible]
7 [illegible] & Sons, Inc
8 [illegible] J f Psychol [illegible]
9 [illegible]
10 [illegible] Zentralbl f Bakt 16 175
11a BUSCHKE, A 1895 Ein fall von chronischem rotz beim menschen Deutsche [illegible]
11b [illegible]
12 [illegible]
13 [illegible]
14 [illegible]
15 BENHAM, R W 1935 Cryptococci—their identification by morphology and by serology J Infect Dis 57. 225
16 COX, L B & J C TOLHURST 1946 Human Torulosis Melbourne University Press Melbourne
17 MARSHALL, M & R W Teed 1942 Torula histolytica meningoencephalitis J A M A 120 527
18 KEENEY, E L, L AJELLO, & E LANKFORD 1944 Studies on common pathogenic

fungi and on Actinomyces bovis II In vitro effect of sulfonamides Bull Johns Hopkins Hosp 76 393
19 Holden M, B Seegal & H Baer 1947 Range of antibiotic activity with protoanemonin Proc Soc Exp Biol & Med 66(1) 54
20 Kutzing F 1833
21 Vuillemin, P 1901
22 Persoon, C H 180
23 Turpin, P J F 183
aceteuse Compt
24 Pasteur L 1876 Etudes sur la biere 387 Gauthier Villars Paris
25 Hansen E C 1888 Undergelser over alkoholgjaersvampenes Medd Lab Carlsb 22 155-310 220-256
26 Berlese A N 18
logia di Cevelino
27 Frothingham, L
New York
Mycopathologia 1(1) 62-67
Anaskosporogenen Hefen Zweite
31 Sanfelice F 1895 Ueber einen neuen pathogen Blastomyceten Centralbl f Bakt I 18 521
32 Todd, R L & W W Herrmann 1936 The life cycle of the organism causing yeast meningitis J Bact 32 89
33 Redaelli
Soc It
34 Goetz C
Abst
35 Emmons
310
36 Mager
Bact
37 Crowe, J T A F de Groat & J G Wahlin 1937 Torula infection Am J Path 13 863
38 Gilchrist T C 1896 A case of Blastomycetic dermatitis in man Johns Hop kins Rep 1 269
39 Montgomery F H & O S Ormsby 1908 Systemic Blastomycosis Arch Int Med 2 1-41
40a Martin D S & D T Smith 1939 Blastomycosis Am Res Tuberc 39 275
40b Martin D S & D T Smith 1936 The laboratory diagnosis of Blastomycosis J Lab & Clin Med 21 1289
41 Sanderson E S & E C Smith 1926 The effect of gentian violet on the organism of blastomycotic infection Arch Dermat & Syph 16 153
42a Noojin R O & J L Callaway 1943 Action of sulfonamide compounds on *Blastomyces dermatitidis in vitro* Arch Dermat & Syph 47 620
42b Noojin R O & J L Callaway 1944 Effectiveness *in vitro* of sulfonamide compounds on *Sporotrichum schenkii* Arch Dermat & Syph 49 305
43 Foster J W & H B Woodruff 1943 Microbiological aspects of streptothricin
44
45
46
47
48
49 Benham R W 1934 The fungi of Blastomycosis and coccicioidal granuloma Arch Dermat & Syph 30 385
50 Ciferri R & R Redaelli 1935 Prima contribuzione allo studio delle cosi dette blastomycosi Americane R Atti dell Instituto Botanico del universita de Pavia 6 55-102

51 DODGE, C W 1935 Medical Mycology C V Mosby St Louis
52 MOORE M 1933 Blastomycosis Report of a case with a study of an etiologic factor and a classification of the organism Annals of the Missouri Bot Gard 20 79
53 HENRICI A T 1947 Molds, Yeasts and Actinomycetes John Wiley & Sons Inc New York
54 RICKETTS, H T 1901 Oidiomycosis (Blastomycosis) of the skin and its fungi Jour Med Res 6(3) 9374-9546
55 LEVINE, S & Z J ORDAL 1946 Factors influencing the morphology of *Blastomyces dermatitidis* J Bact 52(6) 687
56 SPRING, D 1929 Comparison of seven strains of organisms causing Blastomycosis in man J of Infect Dis 44 169

THE NUTRITIONAL REQUIREMENTS OF THE FAVIFORM TRICHOPHYTONS*

By Lucille Katharine Georg

Departments of Dermatology and Bacteriology Columbia University College of Physicians and Surgeons New York N Y

In a recent report Suppurative Ringworm Contracted from Cattle,[1] a series of twenty three cases of deep suppurative ringworm have been described The cases appeared during a three-year period among a community of farmers and their families in central Pennsylvania These unusually severe ringworm infections occurred in order of frequency on the neck face wrists forearms and in the occipital region of the scalp The cases were divided into three groups the sycosis parasitica type, or suppurative ringworm of the bearded areas of the face and neck the kerion type found in the scalp and agminate folliculitis or suppurative lesions of the glabrous skin In all instances there was a history of recent contact with cattle and in all but five cases the patients had observed ringworm lesions on their cattle The lesions their clinical course, and their treatment have been described in detail

Cultural studies indicated that fourteen of these cases had been caused by the large spored faviform trichophytons of the *album discoides* and *ochraceum* varieties and four cases by the common *T mentagrophytes* In the remaining five cases in this series no parasitized hairs were found and no cultures were obtained The nature of the lesions however the absence of bacterial infection and the history of contact with cattle infected with ringworm suggest that these cases also were caused by ringworm contracted from cattle

The faviform trichophytons are characterized by their large spored ectothrix parasitism of the hair and by the extremely slow growing non sporulating type of growth which they produce on the usual laboratory mediums On Sabouraud s dextrose or maltose mediums the cultures are usually of a glabrous type varying from moist heaped up waxy cerebriform colonies to colonies covered with a very fine short white down which appears irregularly on their often highly convoluted surfaces Such colonies are composed of an irregularly branched mycelium similar to that characteristically seen in cultures of *Trichophyton schoenleini* the causative organism of favus Chlamydospores may be present in large numbers but microconidia and macroconidia are rarely produced on these mediums

The poor growth of the faviform trichophytons on the usual mediums makes their isolation from ringworm lesions as well as the maintenance of stock cultures extremely difficult Their inability to produce spores on the usual mediums has led to great confusion in their classification However when enriched or natural mediums are used microconidia and macroconidia typical of the *Trichophyton* group have been produced[2,3]

In our recent experience in the isolation of faviform trichophytons from

* The author wishes to express her gratitude to Dr Rhoda W Benham of the Department of Dermatology Columbia University College of Physicians and Surgeons, for advice and assistance in this work.

ringworm lesions, it was found that, by the use of a medium rich in peptones such as heart infusion tryptose agar (blood agar base, 'Difco"), not only were the chances of isolation greatly improved, but continued good growth with production of microconidia and macroconidia could be obtained in most instances This was particularly true when thiamine was added to the medium, *and it was recommended that heart infusion tryptose agar* with added thiamine (0 1 mg per 100 cc) be used for the isolation, study and maintenance of these cultures

These findings suggest that these organisms are deficient, that is they are unable to synthesize (at least in sufficient quantities) certain growth factors necessary for the initiation and maintenance of growth and for the production of spores That thiamine may be one of these factors is indicated by the production of rapid growing, vigorous, fluffy colonies with many spores when this vitamin is added to a rich peptone medium The possibility of other growth factors in the heart infusion tryptose medium is also to be considered

It is the purpose of this paper to study this group of "deficient organisms in order that a better understanding of their growth characteristics and requirements may be obtained

History A trichophyton of faviform type was first observed by Sabouraud in 1893[4] He isolated the organism from a child who had a flat lesion on the temple which was suppurating at many points from gummy, cracked crusts The culture developed with extreme slowness and showed characteristics analogous to those of *Achorion favus*, the causative organism of favus now known as *Trichophyton schoenleini* Preparations of the diseased hairs however, did not reveal an endothrix parasitism, as seen in favic hairs, but they did show an ectothrix fungus with large spores in mosaic and occasional chains of spores lying along the side of the hair The radical part of the hair was filled with branching mycelium The spores varied in size from 5 to 8 microns

E Bodin who studied this organism, was also able to isolate several similar cultures from lesions on horses, a donkey, and a calf Bodin[5] has furnished a description of a species which he called "*Trichophyton faviforme du veau*' (it was later named *T verrucosum* [1902]) ' On glucose agar the culture forms a little cake in a month's time Part is submerged in the agar and the surface is irregular, verrucose and grey It may show a central accumulation ' In the epidemic among horses reported by Bodin the hairs showed that the parasite was an ectothrix fungus He also observed the infection in nine persons and described the human lesions as a 'thick kerion in the form of a cupola riddled with suppurating follicles

In 1908 Sabouraud[6] described two species in this group One, which he called *Trichophyton ochraceum*, corresponds well to the *T verrucosum* described by Bodin except that it is a brilliant yellow ochre The second species *T album* is difficult to distinguish from *T schoenleini* Sabouraud described it on 3 per cent peptone medium as a glabrous waxy colony of spongy surface and vermicellular appearance On maltose agar, it is heaped up and shows an umbilicate center and sloping sides which have many

irregular folds The texture is tough and rubbery The cultures were extremely slow growing and mediocre in development compared to favus cultures He described lesions of four types caused by these organisms (1) typical kerion lesions with considerable subcutaneous suppuration (2) plaques resembling seborrheic eczema (3) typical ringworm lesions of the annular type with cleared center and vesicular outer border and (4) young lesions which were dry and leathery in appearance

In 1910 Sabouraud[8] described a fourth species *Trichophyton discoides* which he stated was somewhat similar to *T album* except that its growth was almost a perfect disc with a flat surface and often a central knob The whole growth had a light tan to brown color with a moist surface The cultures may develop a fine short, white down This organism was habitually found in cattle and produced lesions of the kerion type in man

Sabouraud who used only sugar mediums with small amounts of peptones observed only the slow growing glabrous faviform colonies described above He did not feel however that the appearance of the cultures was of enough importance to classify the organisms among the *Achorions* and although unable to show any structures characteristic of trichophytons he placed them in the *Trichophyton* group on the basis of the large-spored ectothrix parasitism of the hair and the tinea like lesions produced in man as well as in the experimental animal

Many workers studied the organisms of the faviform group in an attempt to obtain spore-bearing structures which would more clearly identify them as members of the *Trichophyton* group The introduction of the natural mediums of polysaccharide base by Langeron and Milochevitch[7] greatly aided this study On these mediums which consisted of whole grains of wheat barley corn and oats it was found that the faviform trichophytons developed quite rapidly and were very different in appearance from the glabrous colonies obtained on the sugar mediums The growth was of a white velvety to fluffy character similar to the growth of the more common trichophytons Microscopic examination revealed microconidia characteristically borne by the hyphae in clusters (*en grappe*) and laterally (*en thyrse*) Also rudimentary spirals and macroconidia typical of the *Trichophyton* group were demonstrated

In 1934 Lebasque[4] carefully reviewed the faviform group and was able by the use of the polysaccharide mediums to complete the morphological studies of the species already known as well as three new species Of the large number of species which had been described by various workers on the basis of the gross appearance of the colonies Lebasque accepted only six as having characteristics sufficiently distinct to be considered as different species *T verrucosum* (Bodin 1902) *T ochraceum* (Sabouraud 1902) *T album* (Sabouraud 1909) *T discoides* (Sabouraud 1910) *T equinum* (Gedoelst 1902) and *T caballinum* (Neveu Lemaire 1921) He also described three new species *T bullosum* *T villosum* and *T papillosum* For all these species Lebasque was able to obtain cultures which showed all the macroscopic and microscopic characteristics typical of the *Trichophyton* group He described the parasitism of the hair by these species as endo-ectothrix because of the vigorous development of the flexous

ringworm lesions it was found that, by the use of a medium rich in peptones such as heart infusion tryptose agar (blood agar base Difco) not only were the chances of isolation greatly improved, but continued good growth with production of microconidia and macroconidia could be obtained in most instances This was particularly true when thiamine was added to the medium and it was recommended that heart infusion tryptose agar with added thiamine (0 1 mg per 100 cc) be used for the isolation stud and maintenance of these cultures

These findings suggest that these organisms are deficient that is they are unable to synthesize (at least in sufficient quantities) certain growth factors necessary *for the initiation and maintenance of growth and for the* production of spores That thiamine may be one of these factors is indicated by the production of rapid growing vigorous fluffy colonies with many spores when this vitamin is added to a rich peptone medium The possibility of other growth factors in the heart infusion tryptose medium is also to be considered

It is the purpose of this paper to study this group of deficient organisms in order that a better understanding of their growth characteristics and requirements may be obtained

History A trichophyton of faviform type was first observed by Sabouraud in 1893[4] He isolated the organism from a child who had a flat lesion on the temple which was suppurating at many points from gummy cracked crusts The culture developed with extreme slowness and showed characteristics analogous to those of *Achorion favus* the causative organism of favus now known as *Trichophyton schoenleini* Preparations of the diseased hairs however, did not reveal an endothrix parasitism as seen in favic hairs but they did show an ectothrix fungus with large spores in mosaic and occasional chains of spores lying along the side of the hair The radical part of the hair was filled with branching mycelium The spores varied in size from 5 to 8 microns

E Bodin who studied this organism was also able to isolate several similar cultures from lesions on horses a donkey and a calf Bodin[5] has furnished a description of a species which he called *Trichophyton faviforme du veau* (it was later named *T verrucosum* [1907]) On glucose agar the culture forms a little cake in a month s time Part is submerged in the agar and the surface is irregular, verrucose and grey It may show a central accumulation In the epidemic among horses reported by Bodin the hairs showed that the parasite was an ectothrix fungus He also observed the infection in nine persons and described the human lesions as a thick kerion in the form of a cupola riddled with suppurating follicles

In 1908 Sabouraud[6] described two species in this group One which he called *Trichophyton ochraceum* corresponds well to the *T verrucosum* described by Bodin except that it is a brilliant yellow ochre The second species *T album* is difficult to distinguish from *T schoenleini* Sabouraud described it on 3 per cent peptone medium as a glabrous waxy colony of spongy surface and vermicellular appearance On maltose agar it is heaped up and shows an umbilicate center and sloping sides which have many

irregular folds The texture is tough and rubbery The cultures were extremely slow growing and mediocre in development compared to favus cultures He described lesions of four types caused by these organisms (1) typical kerion lesions with considerable subcutaneous suppuration, (2) plaques resembling seborrheic eczema, (3) typical ringworm lesions of the annular type with cleared center and vesicular outer border, and (4) young lesions which were dry and leathery in appearance

In 1910, Sabouraud[6] described a fourth species, *Trichophyton discoides*, which he stated was somewhat similar to *T album*, except that its growth was almost a perfect disc with a flat surface and often a central knob The whole growth had a light tan to brown color with a moist surface The cultures may develop a fine, short white down This organism was habitually found in cattle and produced lesions of the kerion type in man

Sabouraud, who used only sugar mediums with small amounts of peptones observed only the slow growing glabrous faviform colonies described above He did not feel however that the appearance of the cultures was of enough importance to classify the organisms among the *Achorions*, and, although unable to show any structures characteristic of trichophytons he placed them in the *Trichophyton* group on the basis of the large spored ectothrix parasitism of the hair and the tinea like lesions produced in man as well as in the experimental animal

Many workers studied the organisms of the faviform group in an attempt to obtain spore bearing structures which would more clearly identify them as members of the *Trichophyton* group The introduction of the natural mediums of polysaccharide base,[7] by Langeron and Milochevitch,[7] greatly aided this study On these mediums which consisted of whole grains of wheat, barley corn and oats it was found that the faviform trichophytons developed quite rapidly and were very different in appearance from the glabrous colonies obtained on the sugar mediums The growth was of a white velvety to fluffy character similar to the growth of the more common trichophytons Microscopic examination revealed microconidia characteristically borne by the hyphae in clusters (*en grappe*) and laterally (*en thyrse*) Also rudimentary spirals and macroconidia typical of the *Trichophyton* group were demonstrated

In 1934 Lebasque[8] carefully reviewed the faviform group and was able by the use of the polysaccharide mediums to complete the morphological studies of the species already known as well as three new species Of the large number of species which had been described by various workers on the basis of the gross appearance of the colonies Lebasque accepted only six as having characteristics sufficiently distinct to be considered as different species *T verrucosum* (Bodin 1902) *T ochraceum* (Sabouraud 1902) *T album* (Sabouraud 1909) *T discoides* (Sabouraud 1910) *T equinum* (Gedoelst 1902) and *T caballinum* (Neveu Lemaire, 1921) He also described three new species *T bullosum* *T villosum* and *T papillosum* For all these species Lebasque was able to obtain cultures which showed all the macroscopic and microscopic characteristics typical of the *Trichophyton* group He described the parasitism of the hair by these species as endo-ectothrix because of the vigorous development of the flexous,

dichotomously dividing mycelium in the interior of the hair This picture, however except for the larger size of the spores, is not different from that occasionally seen in microsporum infections, where the interior of the hair may be filled with branching mycelium The presence of mycelial filaments in the interior of the hair, as described by Baudet[8] and often seen by us in examination of hairs from cattle as well as from patients, probably is indicative only of the early stages of invasion by the fungus There seems to be no valid reason for calling the parasitism endo-ectothrix

One of the species studied by Lebasque, *Trichophyton equinum* (Gedoelst), presented a downy to fluffy culture on the classic "proof medium" However, spores were not obtained until the culture was grown on the natural carbohydrate mediums Lebasque did not feel that there should be a

members of the group as had

9

e vitamin-deficient organisms was clearly shown by Robbins, Mackinnon, and Ma[10] in their study of the Mackinnon strain of *T discoides* isolated in Uruguay They demonstrated that this strain required three vitamins for growth pyridoxine, i inositol, and molecular thiamine They were able to produce growth on a basal medium prepared with purified agar, recrystallized asparagine dextrose phosphate buffers, and inorganic salts only after these three vitamins had been added The thiamine could not be substituted for by an equal mixture of thiazole and pyrimidine

Burkholder and Moyer,[11] have shown that a strain which they call *Trichophyton faviforme* has a deficiency for thiamine and inositol, and that its growth is stimulated by media containing liver and peptones

Schopfer and Blumer,[12] in their study of the growth requirements of a strain of *T album* which they isolated, have shown that this particular strain, although able to grow to some extent on a basal medium without the addition of any vitamins, produced a more rapid growth when biotin was added This was particularly true when a nitrogen source such as ammonium citrate was used in place of asparagine or amino acids The action of the biotin was shown to be dependent on the physiological age of the culture They also demonstrated that, in an unbuffered nutrient medium, thiamine, inositol, and pyridoxine clearly furthered the development when asparagine was used as a nitrogen source Here it was found

for the

iting

a 1

they

obtained a mycelial weight three times greater than in control flasks

In contrast to these studies, *T schoenleini* has never been shown to be stimulated by vitamins That this is also a deficient organism, however, has been shown by the following findings (1) Whole grain mediums stimulate the production of rapidly growing, heavily powdery or downy cultures with numerous microconidia This is in contrast to the usual slow growing glabrous cultures obtained on the routine sugar mediums, which rarely

show any spores (2) Catanei[13] observed that cultures contaminated by bacteria, staphylococci, and certain bacilli, present a powdery or downy aspect in contrast to the glabrous colonies of pure isolates This has been shown to be due to a water soluble, heat stable substance produced by the growth of the bacteria

Experimental

Part I Cultural and Morphologic Studies of Recently Isolated Faviform Trichophytons

Eight strains of faviform trichophytons recently isolated from suppurative ringworm lesions[1] were selected for cultural and morphologic studies Pure strains were obtained by isolating six single spores from each strain (according to the method described by Georg[14]) Cultures were made on the following mediums (1) simple sugar mediums—Sabouraud s dextrose and maltose agar ("Difco") and Sabouraud s maltose "proof medium" prepared with crude maltose and French peptone according to the method described by Sabouraud[6] (2) whole grains—moist rice and barley prepared according to the method of Langeron and Milochevitch[7], and (3) enriched mediums—dextrose and maltose agar enriched with yeast extract liver extract beef heart infusion Bacto-tryptose, and citrated human blood Cultures were grown both at room temperature and at 37°C on moist and dry mediums of pH varying from 5 0 to 7 4

A Cultural Characteristics on the Simple Sugar Mediums

1 Gross Appearance On Sabouraud s dextrose and maltose agar ("Difco") the different strains produced colonies which showed considerable variation from one another even on the same medium These ranged from completely glabrous moist heaped cerebriform colonies the color of wax to irregularly folded or completely flat disc shaped colonies covered with a short white down and glabrous highly verrucose colonies of a bright yellow ochre color

On first isolation or early transplantation to these mediums, all colonies tended to be glabrous and of a tough leathery consistency After prolonged cultivation and repeated transplants however, the large majority developed a fine white powder at the base of their acuminate structure or in the crevasses of their folds Still other isolates which presented a flatter colony often with a central umbo and a scalloped or irregular border, developed a short white down which covered the entire colony A third type of colony developed by two of the strains began as a moist, colorless, skin like growth over the surface of the medium This later developed glabrous projecting buttons which were a bright yellow ochre Often the whole surface of the colony became highly verrucose and bright yellow ochre These cultures usually developed some fine white powder at the periphery and in the center of the colony which when dense, gave a powdery gray central area and border to the otherwise yellow ochre to deep orange colonies (FIGURE 1)

In general, growth on these mediums was very slow and meager at room

FIGURE 1 *Tr chophyton fav fo me*—Sabouraud s dextrose agar (D f o) 40 days (a) varie y *album* (b) va ety *d o des* (c) va ety *och a eum*



F GURE 2 *T hophyton fav fo me*—Sabouraud s dextrose agar (D f o) Chara te st c morphology show ng r egular bran hed fav fo m my el um w h nume ous ch amydospo es 400X

temperature At 37°C the rate of growth was considerably accelerated but even under these conditions the growth did not usually appear until the fifth to seventh day On the average colonies grown at room temperature approximated 30 millimeters in diameter after 6 weeks of growth They were however, often five to ten millimeters in height in the center The optimum pH was found to lie between 6 8 and 7 2

In order to identify these colonial types more clearly with those described by Sabouraud and other workers the cultures were planted on Sabouraud s maltose proof medium On this medium growth was more rapid and colonies attained a greater size In general the colonies were more heaped and folded and tended to develop more powder and down than on the Difco mediums The colonial types were the same however

2 *Microscopic Appearance* Microscopic examination of the cultures developed on Sabouraud s maltose and dextrose agars both the Difco product and the original proof medium revealed an irregular branched mycelium with an abundance of intercalary and terminal chlamydospores (FIGURE 2) The appearance in some cases was very similar to the favic chandeliers produced by *T schoenleini* In other cases, the mycelium was less irregular and only produced occasional clubbed ends and intercalary chlamydospores Reproductive spores microconidia and macroconidia were characteristically absent

3 *Variation* In general three types of colonies predominated which corresponded rather well with the three species described by Sabouraud as *T album T discoides* and *T ochraceum*[6] After a series of transplants over a period of several months however it soon became evident that these colonial forms were not stable and that a single culture could produce all three of these colonial varieties In order to determine the nature and frequency of these changes a series of studies was made using several single spore cultures from each strain and transplanting serially on Sabouraud s dextrose agar and wort agar (Difco)

Each strain tended to maintain a rather characteristic colonial morphology which clearly differentiated it as a variety However all the variations described above were found in six of the strains studied A parent culture might be flat and covered with a fine white down while the subculture would develop into a completely glabrous heaped cerebriform colony or a glabrous flat but highly verrucose colony of the yellow ochre variety Also a single culture on wort agar which started as a completely glabrous colony might in the course of weeks develop a heavy white down around the edge of the colony in isolated tufts or over the entire surface That this was not an instance of the development of pleomorphic growth commonly seen in some trichophyton cultures was shown by the fact that these fluffy areas were not composed of sterile hyphae but were in fact rich in microconidia The fluffy to glabrous change was also common particularly on the wort agar and a third change back again to the fluffy state was observed in several cultures (FIGURES 3 and 4) Some strains presented both the heaped glabrous and the flat downy type variation in a single colony (FIGURE 5)

FIGURE 1. *Tr chophyton fav forme*—Sabouraud s dextrose agar (D fco) 40 days (a) var ety *album* (b) var ety *di coides* (c) var ety *ochraceum*



FIGURE 2. *Tri hophyton favsforme*—Sabouraud s dextrose agar (D fco) Characterist c morphology show ng irregular branched fav form mycel um w th numerous chlamydospores 400X

temperature At 37°C, the rate of growth was considerably accelerated, but, even under these conditions, the growth did not usually appear until the fifth to seventh day On the average, colonies grown at room temperature approximated 30 millimeters in diameter after 6 weeks of growth They were, however, often five to ten millimeters in height in the center The optimum pH was found to lie between 6 8 and 7 2

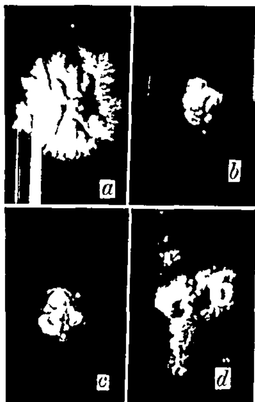
In order to identify these colonial types more clearly with those described by Sabouraud and other workers, the cultures were planted on Sabouraud's maltose "proof medium" On this medium, growth was more rapid and colonies attained a greater size In general, the colonies were more heaped and folded and tended to develop more powder and down than on the "Difco" mediums The colonial types were the same, however

2 *Microscopic Appearance* Microscopic examination of the cultures developed on Sabouraud's maltose and dextrose agars, both the Difco" product and the original "proof medium" revealed an irregular branched mycelium with an abundance of intercalary and terminal chlamydospores (FIGURE 2) The appearance, in some cases, was very similar to the 'favic chandeliers" produced by *T schoenleini* In other cases, the mycelium was less irregular and only produced occasional clubbed ends and intercalary chlamydospores Reproductive spores, microconidia and macroconidia were characteristically absent

3 *Variation* In general, three types of colonies predominated which corresponded rather well with the three species described by Sabouraud as *T album*, *T discoides*, and *T ochraceum* [4] After a series of transplants over a period of several months, however, it soon became evident that these colonial forms were not stable and that a single culture could produce all three of these colonial varieties In order to determine the nature and frequency of these changes, a series of studies was made using several single spore cultures from each strain and transplanting serially on Sabouraud s dextrose agar and wort agar (Difco")

Each strain tended to maintain a rather characteristic colonial morphology which clearly differentiated it as a variety However, all the variations described above were found in six of the strains studied A parent culture might be flat and covered with a fine white down while the subculture would develop into a completely glabrous, heaped cerebriform colony or a glabrous, flat but highly verrucose colony of the yellow ochre variety Also, a single culture on wort agar which started as a completely glabrous colony might, in the course of weeks develop a heavy white down around the edge of the colony, in isolated tufts or over the entire surface That this was not an instance of the development of pleomorphic growth commonly seen in some trichophyton cultures was shown by the fact that these fluffy areas were not composed of sterile hyphae, but were in fact, rich in microconidia The fluffy to glabrous change was also common particularly on the wort agar, and a third change back again to the fluffy state was observed in several cultures (FIGURES 3 and 4) Some strains presented both the heaped glabrous and the flat downy type variation in a single colony (FIGURE 5)

Variation in the faviform trichophytons was first suggested by Cazalbou in 1913[15] when he described a species which he called *Trichophyton singulare*, which had two cultural states, a glabrous cerebriform colony and a downy flat disc-shaped colony. The cultures were found to be reversible. In 1938, Gammel and Work[16] described a case of sycosis parasitica due to a faviform trichophyton which they designated as *Favotrichophyton album*,

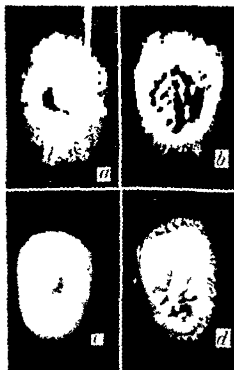


variety *singulare*. After several transplants on liver infusion agar, they obtained two distinct types of growth, a cerebriform type and a less well differentiated discoid type.

These findings, as well as our data obtained with single spore cultures, seem to indicate that *T. album*, *T. discoides*, and *T. ochraceum* are not morphologically distinct and suggest that they are variants of a single species. We propose that they be classified as *T. faviforme* (varieties *album*, *discoides*, and *ochraceum*).

B Growth on Whole Grains

1 Gross Appearance On the natural polysaccharide mediums, moist



extended from the kernels and was visibly erect In some cultures true fluffy growth appeared

2 Microscopic Appearance The mycelium is well developed in 12 to 15 days and consists of a regular branching flexuous mycelium with occasional chlamydospores The aerial growth contains numerous conidia which are seen scattered along the mycelium *(en thyrse)* as well as in terminal pine tree like clusters *(en grappe)* (FIGURES 6 and 7) These microconidia are not pediculated and break off squarely from their point of attachment

to the mycelium on which they are implanted perpendicularly They are easily detached and are sprinkled all through the preparation Their size varies from 1 5 to 2 microns by 3 to 4 microns Although there is considerable variation in size and shape in general they tend to be more slender



F GURE 5 Colony showing both a bum and d scoid variat on Deve oped on Sabouraud s dextrose aga from a s ngle spore culture orig nally of the a bum variety



FIGURE 6 *Trichophyton faviforme* from g owth on rice g a ns M ocon a en thy se" 400 X

and elongated than the typical pear shaped or round conidia of the gypseum group

Macroconidia are also found in the downy to fluffy aerial mycelium appearing from the tenth to thirtieth day These range from 20 to 30 microns



F G 88 ... bo m Trichop ... T ichoph ... om gr w h ... un grow h ... gra n ... gra ns ... rocon dia ... M croconid ... 400X

by 5 t 8 m rons and l r e to 8 t n They occur e ther term nally or lterall u ually s ngly at th en l f a n cel al branel Some are short and bu b l ke others sho long po n e l en ls and others are rregul rly s ollen and l sto ted (F URE 8

C Growth on Enriched Mediums

Yeast and liver extracts both had a definite stimulating effect on the growth rate of the organisms when added to the simple sugar mediums On such enriched mediums, all the strains grew rapidly, producing heavy, compact colonies covered with a white, downy to fluffy aerial mycelium which contained microconidia and occasional macroconidia The amount

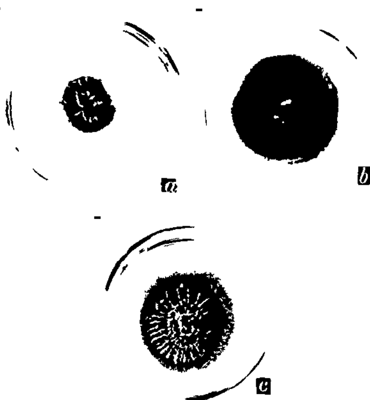


FIGURE 9 *Tri hophyton fav forme*—heart nfus on tryptose agar plus th am ne (0 1 mg per 100 cc) 40 days a) variety *album* (b) var ety *d coides* (c) var ety *ochraceum*

of aerial growth depended on the strain as well as the amount of the extract added

Additions of beef heart infusion, Bacto tryptose and citrated human blood were studied in view of the fact that, in the original isolation work, blood agar plates prepared with beef heart infusion and Bacto tryptose (blood agar base Difco ') had been used and found far superior to Sabou

had been added or not Both the beef heart infusion and the Bacto-tryptose were found to have stimulating effects when added to the simple sugar mediums Either of these substances, added in amounts comparable to that found in the complete medium, caused the development of rapidly growing, vigorous colonies which often reached a diameter of 40 to 60 mm in 4 weeks and were covered with a white powder or a short, erect white down Addition of thiamine (0 1 mg per 100 cc) to the heart infusion tryptose medium further increased the rate of the growth and produced more aerial mycelium, which was, in most cases filled with large numbers of microconidia and occasional macroconidia (FIGURE 9)

Part II Studies of the Vitamin Requirements of the Faviform Trichophytons

A study was made to determine the vitamin requirements of sixteen recently isolated strains of faviform trichophytons as well as of four stock cultures *T album T discoides*, and *T ochraceum*, obtained from the Central Bureau for Fungus Cultures, Delft, Holland, and a strain of *T discoides* isolated in Uruguay, kindly furnished by Dr J Mackinnon The vitamin requirements of this last strain have been studied and reported by Robbins, Mackinnon, and Ma[16] Synthetic, vitamin free mediums were used A basal broth was prepared as follows a mixture of 2 0 gm asparagine (recrystallized 3 times), 0 1 gm $MgSO_4 \cdot 7H_2O$, 50 gm dextrose (C P), and 100 cc Sorenson's phosphate mixture (M/15 KH_2PO_4 and Na_2HPO_4 at pH 7 0) is made to a liter with distilled water and sterilized This medium, designated as "Basal Broth A," with or without 1 5 per cent purified agar (purified according to the method of Robbins[17]) was used as such and in various other forms (modified by substituting other sources of nitrogen for the asparagine) in the following vitamin studies

A Qualitative Studies on Basal Agar with Vitamin Additions

"Basal Agar A" was prepared by adding 1 5 per cent purified agar to the asparagine broth, "Basal Broth A" previously described The final pH of the autoclaved medium varied between 6 8 and 7 0 and maintained this range after the various vitamin additions Filtered vitamin solutions were prepared at the beginning of each experiment, and equal volumes of vitamin solutions, containing vitamins in varying concentrations and combinations were added to tubes containing 10 cc of the melted and partially cooled agar, which were then rotated and slanted

Washed inoculum was prepared from growth in heart infusion broth ("Difco") It was found necessary to wash the mycelium four to five times with sterile distilled water before all traces of the broth medium were removed However, even after many washings a tiny shred of mycelium always carried sufficient nutriment so that a small amount of growth on the control basal agar tubes could be detected This "fuzzing" of the inoculum was found to be advantageous as proof was thus afforded that the inoculum was alive Growth of one millimeter or less was considered negative All cultures were allowed to grow at room temperature for 10 weeks

Twenty strains of faviform trichophytons were tested for ability to grow

on the basal agar as well as on basal agar with various vitamin additions The results indicated that (1) seventeen of the strains had similar vitamin requirements namely inositol and molecular thiamine, (2) one strain #18 required pyridoxine in addition to inositol and molecular thiamine and (3) two strains #19 and #20 showed no essential vitamin requirements growing equally well on the basal agar with or without vitamin additions (For descriptions of these strains consult List of Strains)

1 Seventeen strains which require inositol and thiamine This group includes 16 recently isolated strains as well as a stock strain #17 *T discoides* (Papegaay) obtained from the Central Bureau for Fungus Cultures

only in the presence of inositol Pyridoxine has no effect alone or when

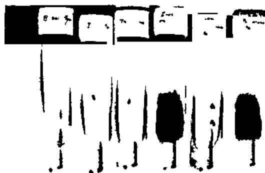


FIGURE 10 *Trichophyton faviforme* strain #11 which requires inositol and thiamine for growth. Addition of pyridoxine and vitamin mixture does not change the amount of growth

added to medium containing super-optimal amounts of inositol and thiamine None of the other vitamins tested included in vitamin *mixture* (calcium pantothenate 100 gamma per cc riboflavin 100 gamma per cc nicotinamide 100 gamma per cc biotin 0 05 gamma per cc para aminobenzoic acid 100 gamma per cc choline chloride 100 gamma per cc and folic acid 100 gamma per cc) showed any effect when tested singly or in all possible combinations or when added with super-optimal doses of inositol and thiamine (FIGURE 10)

Inositol was not effective for certain strains in amounts less than 10 gamma per cc The amount of growth with 10 gamma per cc was very small and for the most part subsurface It was always definitely more growth than on control tubes however and is recorded as one plus Further increase in the inositol did not increase the amount of growth As a result of these findings 100 gamma of inositol (ten times the amount which showed this effect) was used as a super optimal dose to produce the maximum effect of this vitamin in this medium

Thiamine showed no effect alone at a concentration of 0 5 gamma per cc or even when increased to 500 gamma per cc In the presence of a super optimal dose of inositol however 0 5 gamma of thiamine per cc had as much effect as a larger dose of this vitamin, producing a four plus growth of all strains Five gamma of thiamine per cc was taken as a super-optimal dose of this vitamin

2 Strain #18 Which Requires Inositol Thiamine and Pyridoxine Strain #18 *T discoides* (Mackinnon) was shown to require pyridoxine in addition to inositol and thiamine This is in accordance with the findings of Robbins, Mackinnon, and Ma,[10] who studied this strain Inositol showed a very slight effect alone when present in a dosage of 10 gamma per cc or more but neither thiamine or pyridoxine showed any effect unless in the presence of both of the other essential vitamins In the presence of super optimal doses of inositol and thiamine, 0 5 gamma of pyridoxine

TABLE 1

QUALITATIVE GROWTH STUDIES WITH *T faviforme* ON ASPARAGINE BASAL AGAR WITH VITAMIN ADDITIONS

Strains	*Thiamine 5γ/cc*	*Pyridoxine 5γ cc*	*Inositol 100γ cc*	*Inositol & thiamine*	*Inositol thiamine & pyridoxine*	*Thiamine & pyridoxine + vitamin mixture*	*Inositol thiamine & pyridoxine + vitamin mixture*	*Basal agar (control)*
#1-#17	0	0	1+	4+	4+	0	4+	0
#18	0	0	1+	1+	4+	0	4+	0
#19 and #20	4+	4+	4+	4+	4+	4+	4+	4+

(1) Amount of growth is indicated by 1 + extends 2 to 5 mm from inoculum and is largely subsurface and 4 + (heavy growth over the larger part of the slant)
(2) Vitamin mixture gamma per cc pantothenate 100 riboflavin 10 biotin 0 05 and nicotinamide 100 para aminobenzoic acid 1 10 choline 100 and folic acid 100
For descriptions of strains listed consult List of Strains

produced as much effect as a larger dose of this vitamin producing a four plus growth of this strain Five gamma per cc was used as a super-optimal dose None of the vitamins included in the vitamin mixture showed any effect when tested alone or in all possible combinations or when added with super optimal amounts of inositol thiamine and pyridoxine (FIGURE 11)

3 Strains #19 and #20 Which Showed No Essential Vitamin Requirements In contrast to all the other strains #19 *T album* (Baudet and Stuhmer) and #20 *T ochraceum* (Boedijn) stock strains obtained from the Central Bureau for Fungus Cultures Holland were found to have no essential vitamin deficiencies and grew well on the basal agar No stimulation was observed following the addition of inositol thiamine pyridoxine or any of the vitamins included in vitamin mixture

For all of these strains the basal agar alone (or basal agar with supra optimal amounts of required vitamins) was not able to produce growth comparable to that obtained on certain natural mediums Addition of peptones heart infusion Bacto-tryptose yeast or liver extracts to the basal

on the basal agar as well as on basal agar with various vitamin additions. The results indicated that (1) seventeen of the strains had similar vitamin requirements, namely, inositol and molecular thiamine, (2) one strain, #18 required pyridoxine in addition to inositol and molecular thiamine and (3) two strains, #19 and #20, showed no essential vitamin requirements growing equally well on the basal agar with or without vitamin additions (For descriptions of these strains consult, 'List of Strains")

1 Seventeen strains which require inositol and thiamine This group includes 16 recently isolated strains as well as a stock strain, #17, *T discoides* (Papegaay) obtained from the Central Bureau for Fungus Cultures Holland These strains showed morphological and cultural characteristics typical of *T faviforme* of the *album*, *discoides*, and *ochraceum* varieties

For these strains inositol has some effect alone, but thiamine is effective only in the presence of inositol Pyridoxine has no effect alone or when

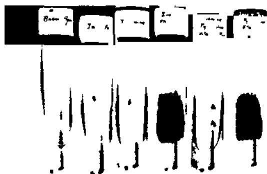


FIGURE 10 *Trichophyton faviforme* strain #11 which requires inositol and thiamine for growth. Addition of pyridoxine and vitamin mixture does not change the amount of growth

added to medium containing super-optimal amounts of inositol and thiamine None of the other vitamins tested, included in 'vitamin mixture' (calcium pantothenate 100 gamma per cc, riboflavin, 100 gamma per cc nicotinamide, 100 gamma per cc, biotin 0 05 gamma per cc, para aminobenzoic acid 100 gamma per cc, choline chloride 100 gamma per cc, and folic acid 100 gamma per cc), showed any effect when tested singly or in all possible combinations or when added with super optimal doses of inositol and thiamine (FIGURE 10)

Inositol was not effective for certain strains in amounts less than 10 gamma per cc The amount of growth with 10 gamma per cc was very small and for the most part subsurface It was always definitely more growth than on control tubes however, and is recorded as one plus Further increase in the inositol did not increase the amount of growth As a result of these findings, 100 gamma of inositol (ten times the amount which showed this effect) was used as a super optimal dose to produce the maximum effect of this vitamin in this medium

Thiamine showed no effect alone at a concentration of 0.5 gamma per cc or even when increased to 500 gamma per cc. In the presence of a super-optimal dose of inositol however 0.5 gamma of thiamine per cc had as much effect as a larger dose of this vitamin producing a four plus growth of all strains. Five gamma of thiamine per cc was taken as a super optimal dose of this vitamin.

2 Strain #18, Which Requires Inositol Thiamine, and Pyridoxine. Strain #18, *T discoides* (Mackinnon), was shown to require pyridoxine in addition to inositol and thiamine. This is in accordance with the findings of Robbins Mackinnon, and Ma [10] who studied this strain. Inositol showed a very slight effect alone when present in a dosage of 10 gamma per cc or more, but neither thiamine or pyridoxine showed any effect unless in the presence of both of the other essential vitamins. In the presence of super optimal doses of inositol and thiamine 0.5 gamma of pyridoxine

TABLE 1

QUALITATIVE GROWTH STUDIES WITH *T faviforme* ON ASPARAGINE BASAL AGAR WITH VITAMIN ADDITIONS

Strains	Thiamine 5γ/cc	Pyridoxine 5γ cc	Inositol 100γ cc	Inositol & thiamine	Inositol thiamine & pyridoxine	Thiamine & pyridoxine + vitamin mixture	Inositol thiamine & pyridoxine + vitamin mixture	Basal agar (control)
#1 #17	0	0	1+	4+	4+	0	4+	0
#18	0	0	1+	1+	4+	0	4+	0
#19 and #20	4+	4+	4+	4+	4+	4+	4+	4+

1) Amount of growth is indicated by 1 + (extends 2 to 5 mm. from inoculum and is largely subsurface) and 4 + (heavy growth over the larger part of the slant)
(2) Vitamin mixture (gamma per cc): pantothenate 100 riboflavin 100 biotin 0.05 nicotinamide 100 para aminobenzoic acid 1 100 choline 100 and folic acid 100
3) For descriptions of strains listed consult List of Strains

produced as much effect as a larger dose of this vitamin. Five gamma per cc was used as a super-optimal dose. None of the vitamins included in the "vitamin mixture" showed any effect when tested alone or in all possible combinations or when added with super-optimal amounts of inositol thiamine and pyridoxine (FIGURE 11).

3 Strains #19 and #20 Which Showed No Essential Vitamin Requirements. In contrast to all the other strains #19 *T album* (Bodin) and #20 *T ochraceum* (Boedijn) stock strains obtained from the Central Bureau for Fungus Cultures Holland were found to have no essential vitamin deficiencies and grew well on the basal agar. No stimulation was observed following the addition of inositol thiamine, pyridoxine or any of the vitamins included in vitamin mixture.

For all of these strains the basal agar alone (or basal agar with supra optimal amounts of required vitamins) was not able to produce growth comparable to that obtained on certain natural mediums. Addition of peptones heart infusion Bacto-tryptose yeast or liver extracts to the basal

agar not only replaced the essential vitamins where these were required but in all cases produced a heavier and more rapid growth This suggests that there are other important nutritional factors required to produce maximum growth

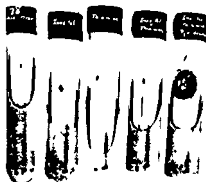


FIGURE 11 *Trichophyton d s oides* (Mack nnon) strain #18 wh ch requ res pyr dox ne in add t on to inos tol and th amine

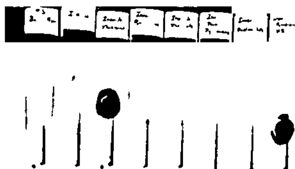


FIGURE 12 *Tri hophyton fariforme* stra n #8 wh ch requ res inos tol and th am ne for growth The th am ne must be p esent n the molecular form and cannot be replaced by th azole pyr m d ne or a comb na t on of these two Oxalacetate shows no act on

4 Influence of Different Nitrogen Sources on the Vitamin Requirements Ammonium chloride 0 5 gm per liter was substituted for asparagine in the basal agar (This medium is designated as Basal Agar B) Also a basal agar was prepared in which 0 05 per cent of vitamin free casein hydrolysate (Smaco) was used in place of asparagine (Basal Agar C) On both of these mediums the essential vitamin requirements of the strains studied were found to be the same as on Basal Agar A,' which contained asparagine as the nitrogen source None of the strains except #19 and #20 would grow on these mediums unless the essential vitamins inositol and thiamine (or inositol thiamine, and pyridoxine in the case of strain #18) had been added

Although all of the strains apparently could utilize the inorganic nitrogen present in the ammonium chloride medium the growth was very scant and did not compare with growth obtained when either asparagine or casein hydrolysate were present

5 Use of Component Parts of the Thiamine Molecule It has been reported[10] that the Mackinnon strain of *T discoides* requires intact thiamine and cannot utilize pyrimidine or thiazole separately or in equimolar combination It was of interest to determine whether the other strains studied in this series were similar in this respect

Pyrimidine, as 2 methyl 5 ethoxy methyl-6 amino pyrimidine and thiazole, as 2 amino thiazole were added singly and in combination, 300 gamma each to the basal agar tubes with inositol Growth on all tubes was very meager and comparable to that obtained with inositol alone Thus, these thiamine requiring strains were shown to need the intact molecule which could not be substituted for by either pyrimidine or thiazole or a combination of the two The experiment was controlled by including in the protocol two thiamine requiring fungi *Phytophthora cinnamomi* which requires the intact molecule and *Phycomyces blakesleeanus* which is able to synthesize thiamine from thiazole and pyrimidine It was also shown that oxalacetate will not substitute for thiamine (FIGURE 12)

B Characteristics of the Growth on Basal Agar with Added Vitamins

As described above none of the vitamin deficient strains produced any growth beyond a slight fuzzing of the inoculum on the vitamin free basal agar with asparagine casein hydrolysate or ammonium chloride as nitrogen source With the addition of inositol 10 gamma per cc a small amount of growth was produced in all cases This was largely submerged and consisted of fine, branching mycelium extending into the medium for a distance of 2 to 5 millimeters

The addition of thiamine to the basal medium containing inositol greatly altered the character as well as the amount of the growth The seventeen strains which required inositol and thiamine produced vigorously growing colonies on this medium The growth was compact and heavy and was generally covered with a thick white fluffy aerial mycelium The mycelium appeared more regular than on Sabouraud s dextrose agar and the number of chlamydospores was greatly reduced The production of reproductive spores varied greatly with the strain All of the strains showed microconidia and in some strains the aerial mycelium was loaded with them (FIGURE 13) On the whole they were more numerous than on rice grains on which medium these structures have been described in detail previously Macroconidia were produced in small numbers by all of the strains on the vitamin-enriched basal agar They were characteristically very small and delicate Two strains produced macroconidia in great abundance on the basal agar with inositol and thiamine These were long and slender with long tapering ends a characteristic bean pod like structure (FIGURE 14)

Strain #18 *T discoides* (Mackinnon) sl ved very little growth on the



FIGURE 13. *Trichophyton faviforme* strain #5 on basal asparagine agar enriched with inositol and thiamine. Note large number of microconidia. 80X

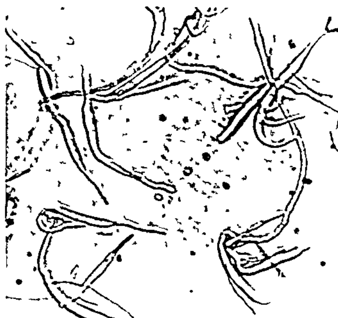


FIGURE 14. *Trichophyton faviforme* strain #8 on basal asparagine agar enriched with inositol and thiamine. Note large number of macroconidia. 400X

basal agar containing inositol and thiamine the amount of growth being the same as when inositol was present alone Addition of pyridoxine, however, to the medium containing supra-optimal doses of inositol and thiamine stimulated this strain to grow vigorously and to produce colonies which were comparable to those produced by the other strains on basal agar with inositol and thiamine Both microconidia and a few irregular macroconidia were seen

Strain # 19 *T album* (Baudet and Stuhmer) grew on the basal mediums without vitamin additions Growth on these mediums however was almost always completely glabrous The colonies were raised and heavily folded frequently being ballooned-out so that the inner areas were hollow The growth was dry and crumbly and consisted of masses of chlamydospores and fragments of irregular faviform hyphae No microconidia or macroconidia were ever seen on this medium Additions of vitamins—large amounts of thiamine inositol and pyridoxine as well as all the vitamins included in 'vitamin mixture —had no effect in increasing the amount or changing the character of this growth

Since this strain was autotrophic for the vitamins in contrast to the heterotrophy exhibited by the strains described above a study was made of its colonial and microscopic morphology to determine whether it could be set apart on this basis also It was found to differ in the following ways (1) it grew at a faster rate producing a thick colony with many foldings which reached a diameter of 80 millimeters in 6 weeks (2) it was habitually covered with a downy white aerial growth even on the simple sugar mediums (3) the growth was only slightly stimulated by the various enrichments added to the sugar mediums and (4) it produced microconidia in greater abundance than did any other strain It seems probable that all of these characteristics may be reflections of the fact that this organism is autotrophic for the vitamins as the other strains show these same characteristics when grown on basal agar supplied with optimal amounts of the vitamins which they require The completely glabrous degenerate type of growth which is produced on basal agar however suggests that although this organism is autotrophic for the vitamins studied it is lacking in ability to produce some other substances present in natural mediums which are necessary for good growth and the production of spores Our results indicate that this deficiency cannot be overcome by large amounts of any of the vitamins tested or by casein hydrolysate

A similar glabrous growth on the basal agar even in the presence of large quantities of vitamins and casein hydrolysate is also characteristic of six strains of *T schoenleini* which we have studied The close relationship of strain # 19 to *T schoenleini* will be discussed later

Strain # 20 *T ochraceum* (Boedijn) also grew well on the basal agars and was not stimulated by any of the vitamins tested Some increase in amount of growth was observed on the enriched mediums On all mediums this strain produced a completely glabrous raised cerebriform to highly verrucose colony which at first appeared a light tan but later developed a grey or dark brown pigment especially in the center of the colony Micro-

scopically it consisted of a faviform type mycelium and large numbers of chlamydospores This strain, obtained from the fungus collection in Holland, has apparently lost the ability to produce the yellow-ochre pigment described by Sabouraud for this species It has also lost the ability to produce a downy culture with spores on whole grain mediums described by Lebasque[8] Although this strain showed no essential vitamin deficiencies there seems to be no reason to consider it as distinct from two strains of *T faviforme* (variety *ochraceum*) which we have isolated which require thiamine and inositol and produce downy cultures with spores on vitamin enriched mediums

C Quantitative Studies in Basal Broth with Vitamin Additions

Using the buffered Basal Broth A " containing dextrose magnesium sulfate, and asparagine several of the strains were studied in order to determine the quantitative effect of addition of the essential vitamins and to compare the maximum growth obtained on vitamin-enriched synthetic medium to that of a natural medium, heart infusion broth (Difco)

The Basal Broth A ' was prepared, adjusted to pH 7 0 and sterilized by filtration Fifty cc amounts were placed in 150 cc Erlenmeyer flasks and the freshly prepared vitamin solutions were added in excess (supra

experiment strains # 1 and # 5 isolated in Pennsylvania and # 17 *T discoides* (Papegaay) a stock strain from Holland On Sabouraud s dextrose agar strain # 1 habitually produced the *album* variety of colony while strain # 5 was of the *discoides* vari

18 *T discoides* (Mackinnon),

inositol and thiamine and strair

a stock strain from Holland which had not shown any vitamin deficiencies

The flasks were inoculated with tiny shreds of washed mycelium prepared as described above, and the cultures allowed to grow at room temperature being shaken at intervals in order to keep the growth of a uniform submerged type After 10 weeks, the entire contents of the flasks were filtered through previously weighed scintered glass filter cups The retained mycelium was washed with at least 200 cc of distilled water and packed into a compact mass on the bottom of the cup The cups were dried in a constant temperature oven at 110°C for two hours and after cooling reweighed on an analytical balance The weight of the mycelium was calculated All glassware was cleaned with acid cleaning solution rinsed well in distilled water and sterilized by dry heat TABLE 2 gives a summary of these tests

None of the vitamin-deficient strains showed any growth in the basal broth beyond a small amount of fuzzing which indicated that the inoculum was alive Strain # 19 produced considerable growth approximately 28 23 mg (average of 6 tests) The single vitamins or the vitamin combinations showed no stimulating effect on this strain and approximately the same

TABLE 2

QUANTITATIVE GROWTH STUDIES WITH *T. Faviforme* IN ASPARAGINE BASAL BROTH WITH VITAMIN ADDITIONS

Strain	*Thiamine* 5γ/cc	*Pyridoxine* 5γ/cc	*Inositol* 100γ/cc	*Inositol & thiamine*	*Inositol, thiamine & pyridoxine*	*Thiamine & pyridoxine + vitamin mixture* *	*Inositol, thiamine & pyridoxine + vitamin mixture* *	*Heart infusion broth*	*Heart infusion broth + inositol thiamine & pyridoxine*	*Basal broth (control)*
#1 (*album* variety) Penna †	0	0	less than 1 mg	14.61 mg	14.39 mg	0	15.96 mg	21.49 mg	39.06 mg	0
#5 (*discoides* variety) Penna †	0	0	1.59 mg	17.51 mg	18.34 mg	0	18.16 mg	45.02 mg	91.70 mg	0
#17 *T. discoides* (Lapegaay) Holland ‡	0	0	1.6 mg	29.17 mg	28.96 mg	0	27.60 mg	48.65 mg	85.15 mg	0
#18 *T. discoides* (Mackinnon) Uruguay †	0	0	1.4 mg	1.35 mg	19.0 mg	0	17.17 mg	17.15 mg	56.85 mg	0
#19 *T. album* (Baudet Stuhmer) Holland ‡	29.18 mg	27.62 mg	30.0 mg	31.19 mg	30.45 mg	27.15 mg	25.20 mg	72.42 mg	73.83 mg	28.23 mg †

* Vitamin mixture—gamma per cc: pantothenate 100, riboflavin 100, biotin 0.05, nicotinamide 100, para-aminobenzoic acid 100, choline 100 and folic acid 100.
† Averages of 6 weighings—dried mycelium from 6 flasks.
‡ Averages of 4 weighings—dried mycelium from 4 flasks.

a larger supply of available nitrogen or the presence of unknown growth factors which cannot be identified with any of the vitamins studied

Growth of the vitamin deficient strains corresponded well with the results obtained on the basal agar slants in the previous experiment The three strains studied which had shown growth with inositol and thiamine #1, #5 and #17, showed even more clearly here that these vitamins were required and that addition of pyridoxine, as well as the other vitamins

basal slants containing this vitamin

Strain #18 was again shown to require pyridoxine in addition to inositol and thiamine The small amount of growth produced by inositol alone was less than 2 milligrams None of the other vitamins tested had any effect on the amount of growth produced

All of the strains showed considerable increase in growth in the flasks containing heart infusion broth However, even more growth could be obtained with the vitamin-deficient strains when super-optimal amounts of the required vitamins (based on the dosages of these vitamins which would produce maximum growth in the basal asparagine medium) were added to the heart infusion broth This indicated that the required vitamins were not present in sufficient amounts to produce maximum growth in this medium As Schopfer and Blumer[15] have shown for *Phycomyces blakesleeanus*, the action of the vitamins is dependent on the medium By increasing the protein content, the optimal dosage of required vitamins is raised and the production of dry matter is increased

Titrations were made in asparagine basal broth in order to determine the smallest amounts of the essential vitamins which would permit growth of several of the strains studied

Basal Broth A was placed in 10 cc amounts in test tubes (150 × 18 mm) and sterilized by autoclaving The vitamin to be titrated was added aseptically in a volume of 10 cc to one of these tubes mixed and serially diluted through a series of 30 tubes the amount of vitamin thus being halved by each transfer Four titrations were carried out for each of the four strains studied and these were done in duplicate (1) titration of inositol alone (2) titration of inositol in the presence of thiamine (1 cc of a standard thiamine solution being added to each tube at the completion of the dilution procedure giving a final concentration of 5 gamma per cc), (3) titration of thiamine in the presence of inositol (1 cc of a standard inositol solution being added to each tube at the completion of the dilution procedure giving a final dilution of 100 gamma per cc), and (4) titration of pyridoxine in the presence of thiamine and inositol Control tubes contained basal broth alone and basal broth with each vitamin singly and in all combinations in supra optimal amounts All tubes were brought to equal volume with sterile distilled water

Strains #1, #5, #18, and #19 were chosen as test organisms Inocula tions were made with minute pieces of washed mycelium and the cultures were allowed to grow at room temperature for 6 weeks The results are summarized in TABLE 3

None of the vitamin deficient strains grew in basal broth control tubes or in tubes containing only thiamine and pyridoxine Some growth oc curred in tubes containing inositol alone The end point of growth was clear cut in all cases and occurred for strain #1 in a dilution containing 75 gamma per cc, for strain #5 at 1 22 gamma per cc and for strain 18 at 4 8 gamma per cc Growth in tubes containing inositol was rep ented by small balls of fluffy growth in the bottoms of the tubes—never more than 2 milligram dry weight Increasing the amount of inositol did not produce a corresponding increase in the amount of growth

TABLE 3

SMALLEST AMOUNTS OF THE ESSENTIAL VITAMINS WHICH WILL ALLOW THE GROWTH OF *T faviforme* IN ASPARAGINE BASAL BROTH

Strain	*Inositol*	*Thiamine (in the presence of excess inositol 100γ cc)*	*Pyridoxine (in the presence of excess inositol and thiamine—5γ/cc)*
	gamma per cc	gamma per cc	gamma per cc
#1	9 75	0 00?	no effect of pyridoxine observed
#5	1 22	0 004	no effect of pyridoxine observed
#18	4 8	no effect of thiamine observed	0 002
#19	no effect of inositol observed	no effect of thiamine observed	no effect of pyridoxine observed

For strains #1 and #5, the presence of super-optimal amounts of thiamine greatly increased this growth, and the same dilutions of inositol which had shown only a small fluffy ball at the bottoms of the tubes were filled with mycelium Growth fell off sharply however at the same dilutions of inositol as it had when inositol had been present alone The action of inositol seems to be concerned with the initiation of growth and development of the mycelium to a certain point only In the presence of thiamine the same minimal amounts of inositol are required to initiate the growth but the development of the mycelium continues with greater utilization of the medium

No growth of any of the strains was obtained with thiamine alone even in dilutions containing 62γ gamma per cc In the presence of supra-optimal doses of inositol, however, heavy growth of strains #1 and #5 occurred in all tubes containing 0 02 gamma or more thiamine per cc In succeeding dilutions, which contained less and less thiamine the growth could be seen

to decrease gradually in amount, the end point of the effect of this vitamin occurring in dilutions containing 0 002 and 0 004 *gamma of thiamine per* cc Beyond these dilutions, only a small amount of growth occurred in all tubes which could be accounted for by the inositol present, which as shown above, has some action on these strains without thiamine Thus thiamine alone showed no action on the growth of these strains But, in the presence of inositol, the amount of growth was roughly proportional to the amount of thiamine present until a maximum amount of growth for this medium was obtained

Titration of pyridoxine in the presence of inositol and thiamine did not give any evidence of stimulation by this vitamin except for strain #18 which has been shown to require pyridoxine[10] With this strain heavy

[illegible]

the effect of this vitamin occurring in a dilution containing [illegible] pyridoxine per cc The very small amounts of growth which occurred in all tubes beyond this dilution could be accounted for by the inositol present, which has some effect by itself

Strain #19, which had not been shown to be vitamin-deficient by previous methods grew equally well in all tubes, thus showing no stimulation by any of the vitamins tested This strain was also tested using dilutions of biotin in the Basal Broth A" and also in ' Basal Broth B ' in order to determine whether biotin had any effect as reported by Schopfer and Blumer[12] for a strain of *T album* No effect of biotin, either in asparagine basal broth or in ammonium chloride basal broth, was observed

Part III Growth Characteristics of Other Species in the Faviform Trichophyton Group

A brief survey was made of other species in the faviform trichophyton group in an attempt to relate these forms on the basis of their cultural characteristics and nutritional requirements The following strains were studied (1) six strains of *T schoenleini* recently isolated from cases of favus and (2) several cultures obtained from the Central Bureau for Fungus Cultures, Holland *T immergens* (Milochevitch), *Favotrichophyton decipiens* (Boedijn and Verbunt), *T bullosum* (Lebasque) and *T equinum* (Gedoelst)

A Trichophyton schoenleini

The six strains of *T schoenleini* studied showed cultural characteristics typical of those described for this species On Sabouraud s dextrose and maltose agar, these strains produce slow growing, heaped and folded colonies which are at first largely glabrous, but later usually develop some white powder and in some areas a short white down Irregular branching mycelia grow in the depths of the medium and form ragged edges about the raised colonies The colony type is variable, a single strain showing reversible changes from completely glabrous to downy colonies re

sembling, in this respect, the colonial variations shown for single spore cultures of *T faviforme* (var *album*, *discoides*, and *ochraceum*) Bacterial contamination of the medium, particularly with the staphylococcus, stimulates the growth of *T schoenleini* so that more white powder and down are obtained Growth on mediums enriched with heart infusion, Bacto-tryptose, yeast, or liver extracts caused some increase in the amount of growth, the colonies being heavier and more folded and often showing a raised, regular border Microscopically, the growth on both the Sabouraud's dextrose agar and the enriched mediums was composed of thick, irregular, highly branched mycelium ("chandeliers") and many chlamydospores Microconidia were extremely rare, except on rice grains, where they were found in small numbers They were small and pyriform and appeared similar to those seen in other trichophyton species Macroconidia were not observed on any medium

All six strains of *T schoenleini* were able to grow on basal agars with ammonium chloride, asparagine, or casein hydrolysate as nitrogen source On these basal agars, they produced completely glabrous, much folded, ballooned-out colonies which became tan to dark brown with age and were dry and crumbly They consisted of irregular fragments of twisted hyphae and many highly irregular chlamydospores The amount of growth on the basal mediums varied considerably with the strain two of which grew rather feebly on these mediums Addition of large amounts of the vitamins studied had no effect in changing the character or increasing the amount of the growth of any of these strains Although these strains of *T schoenleini* appear to be completely autotrophic for the vitamins studied, the completely glabrous, degenerate, and in some instances very meager growth which several of the strains produce on the basal agars suggests that they are lacking in ability to produce some other substances present in natural mediums which are necessary for good growth and spore production (FIGURE 15)

In all respects, except for their slower and usually more glabrous growth and poor ability to produce microconidia, these *T schoenleini* strains resembled quite closely strain #19, *T album* (Baudet and Stuhmer) previously described

B *Trichophyton immergens*

T immergens is described by Milochevitch[19] as follows On Sabouraud's glucose medium it produces an irregular greyish colony with elevated center and is covered with a short, scant white down Around the center there is a large zone of immerging rays, large and uneven The reverse side of the colony is a bright, clear yellow , On maltose agar a fluffy white aerial growth is described The aerial growth consists of sterile mycelium and the submerged growth shows a faviform hyphae and many chlamydospores By growing the cultures on kernels of corn, Milochevitch obtained microconidia, *en thyrse* and *en grappe* but no macroconidia The lesions produced are usually of a deep suppurative type, but typical favic scutulae have been described in several cases of infection due [illegible] anism

The strain of *T immergens* obtained from the stock collection in Holland produced colonies similar to those described by Milochevitch. No stimulation of growth was observed on enriched mediums and no microconidia or macroconidia were obtained, the strain appearing to have become completely pleomorphic The growth on the basal agars was similar in all respects

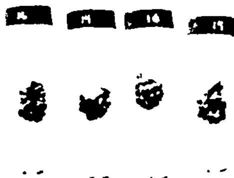


FIGURE 15 *Trichophyton schoenleini* tube #16—growth on basal asparagine agar tube #14—growth on basal asparagine agar plus inositol thiamine and pyridoxine tube #10—growth on basal asparagine agar plus "vitamin mixture" tube #17—growth on basal asparagine agar plus inositol, thiamine, pyridoxine and "vitamin mixture"



FIGURE 16 *Trichophyton equinum* (Boedijn) requires nicotinic acid for growth This can be substituted for by nicotinic amide or l tryptophane

to the growth on both the simple sugar mediums as well as on the enriched mediums Thus, no deficiencies could be detected in this strain

C *Favotrichophyton decipiens*

Favotrichophyton decipiens (Boedijn and Verbunt), also obtained from the fungus collection in Holland, produced a flat, greyish colony with a small raised center and many fine radial grooves The colony was covered with a scant but long greyish decumbent aerial mycelium Microscopically

it consisted of a faviform mycelium and many chlamydospores No stimulation of growth was observed on enriched mediums and no microconidia or macroconidia were obtained—this strain apparently had also become pleomorphic The growth on both the basal agars was similar in all respects to the growth on both the simple sugar mediums as well as on the enriched mediums Thus, this strain also showed no apparent deficiencies

D *Trichophyton bullosum*

T bullosum was described by Lebasque[4] as a very slow growing glabrous culture with a highly irregular surface tending to show dome-shaped protrusions Microscopically it consisted of faviform hyphae and many chlamydospores Lebasque obtained downy cultures on the natural mediums and both microconidia and macroconidia were described

The strain of *T bullosum* obtained from the fungus collection in Holland produced cultures resembling those described by Lebasque It never formed a downy culture, however and no microconidia or macroconidia were obtained on enriched or whole grain mediums This strain would not grow on any of the basal agars and was not stimulated by large doses of any of the vitamins used in these studies Further study is being made to determine the essential growth requirements of this organism

E *Trichophyton equinum*

T equinum (Gedoelst) is described by Lebasque[4] as a large spored ectothrix organism obtained from cattle or horses which produces a downy to fluffy culture on the usual laboratory mediums The fluffy growth as in the case of *T immergens* consists of sterile hyphae By growing the culture on whole grains Lebasque obtained large numbers of microconidia and macroconidia in the 12 strains which he studied

The strain of *T equinum* obtained from the fungus collection in Holland showed a white fluffy growth of sterile mycelium on the simple sugar mediums, as well as on the enriched mediums and produced a bright yellow pigment on the reverse side of the colony Only a few microconidia and an occasional macroconidium were observed on rice grains The organism would not grow on Basal Agars A and B which contained asparagine and ammonium chloride However a very small amount of submerged growth was obtained on the Basal Agar C which contained casein hydrolysate By adding various vitamin solutions to the basal mediums it was found that good growth occurred when either nicotinic acid or nicotinamide was added Growth on the nicotinic acid-enriched basal agars was white and fluffy and composed of sterile hyphae thus resembling the growth of this organism on other mediums No stimulation of spore production or further stimulation of growth was obtained by adding large doses of inositol thiamine pyridoxine or any of the vitamins included in "vitamin mixture" to the basal agars enriched with nicotinic acid Also none of these substances could substitute for nicotinic acid or nicotinamide

The fact that a small amount of growth occurred on Basal Agar C suggested that either the casein hydrolysate was contaminated with small

amounts of nicotinic acid or one of the amino acids present in casein might have stimulating properties for this organism It was found that l trypto phane had this property, and that when added to Basal Agars A or B good growth of *T equinum* could be obtained even in the absence of nico tinic acid or nicotinamide (FIGURE 16) d Tryptophane was found to be inactive The type of growth obtained with l tryptophane was similar in all respects to that obtained when nicotinic acid was present The com bination of nicotinic acid and tryptophane did not change the character of this growth

Titrations were made in ammonium chloride basal broth to determine the smallest amount of nicotinic acid nicotinamide, or l tryptophane which would permit the growth of the strain of *T equinum* studied Results of the titrations were as follows (1) both nicotinic acid and nicotinamide were

show a possible interchangeable role of these two substances Later ex periments by Rosen *et al*[21] and those of Sarett and Goldsmith[22] indicated that tryptophane may be an important precursor of nicotinic acid in rats as well as in humans and may explain the antipellagragenic activity of certain foods such as milk, which is low in nicotinic acid but rich in protein Bonner and Beadle[23] have recently described three X ray mutants of neuro-spora which require nicotinic acid for growth On the basis of their studies Woolley[24] has postulated that, if tryptophane is a precursor of nicotinic acid in neurospora, it must be three enzymatic steps removed from the vita min

According to Schopfer[25] no naturally occurring fungi have been reported to require nicotinic acid Further strains must be obtained and studied before it can be determined whether this requirement reported here for a strain of *T equinum* is characteristic for this species

Summary and Conclusions

(1) As a result of morphological and cultural studies employing single spore strains of recently isolated faviform trichophytons, as well as a study of the growth requirements of these strains it seems apparent that *T album discoides* and *ochraceum* are variants of a single species We propose that they be classified as *T faviforme* (varieties *album, discoides* and *ochraceum*)

(2) On the usual sugar mediums growth was very slow and meagre and of the glabrous nonsporulating type characteristic of the faviform group However cultures on enriched and whole grain mediums demonstrated

that these strains could produce vigorously growing downy to fluffy colonies with a regular mycelium and microconidia and macroconidia characteristic of the *Trichophyton* group

(3) Synthetic, vitamin free mediums were used as a basis for studies of the vitamin requirements of the recently isolated as well as stock strains of *T faviforme* (var *album discoides* and *ochraceum*) A group of closely related species were included in these studies One may postulate that a gradual loss of synthetic abilities has occurred in this group of organisms as suggested by the following findings

(*a*) Autotrophic strains such as *T immergens* (Milochevitch) and *Favotrichophyton decipiens* (Boedijn and Verbunt) two members of the group which produce slightly downy cultures on the usual mediums grow as well on chemically defined vitamin free mediums as on natural mediums However these strains require certain substances present in natural products in order to produce spores This deficiency cannot be overcome by large amounts of any of the vitamins studied or by casein hydrolysate

(*b*) Autotrophic strains such as six strains of *T schoenleini* studied and two stock strains *T album* (Baudet and Stuhmer) and *T ochraceum* (Boedijn) although able to grow to some extent on synthetic vitamin free mediums, produce completely glabrous degenerate colonies on these mediums On certain natural mediums they produce a more luxuriant regular growth with aerial mycelium and spores Here again the deficiency cannot be overcome by large amounts of any of the vitamins studied or by casein hydrolysate

(*c*) A partially heterotrophic strain classified as *T album* has been shown by Schopfer and Blumer[illegible] to have the ability to grow very slightly in a vitamin free medium They found however that its growth could be greatly stimulated by a combination of biotin inositol thiamine and pyridoxine They were able to substitute a combination of pyrimidine and thiazole for the thiamine growth factor

(*d*) A heterotrophic strain *T equinum* (Gedoelst) was found to require one vitamin, nicotinic acid or the closely related compound nicotinamide in order to grow in a synthetic vitamin free medium with either ammonium chloride or asparagine as nitrogen source The vitamin requirement however can be dispensed with in the presence of l tryptophane This substitution of the amino acid for nicotinic acid or vice versa suggests either that tryptophane may be a precursor of nicotinic acid in the metabolism of this fungus or that nicotinic acid may function in some enzyme system necessary for the production of tryptophane Further strains must be obtained and studied before it can be determined whether this requirement is characteristic of the species *T equinum*

(*e*) Heterotrophic strains such as the sixteen recently isolated cultures of *T faviforme* of the *album discoides* and *ochraceum* varieties as well as a stock strain *T discoides* (Papegaay) were shown to require two vitamins inositol and thiamine in order to grow in synthetic vitamin free mediums with ammonium chloride asparagine or casein hydrolysate as nitrogen source The thiamine could not be substituted for by pyrimidine or thiazole

or a combination of these two component parts of the thiamine molecule Inositol produced some effect alone, and seemed to be concerned with the initiation of growth and development of the mycelium only to a certain point Thiamine showed no action alone, but, in the presence of an adequate supply of inositol, it stimulated these strains to produce rapidly growing colonies covered with a thick white aerial mycelium which contained micro

on synthetic, vitamin free medium with asparagine as nitrogen source These are inositol, thiamine, and pyridoxine The thiamine could not be substituted for by pyrimidine or thiazole or a combination of these two component parts of the thiamine molecule These authors also showed that certain other substances present in natural mediums have a stimulating effect on this strain

(g) A heterotrophic strain, *T bullosum* (Lebasque), would not grow on any of the synthetic vitamin free mediums and was not stimulated by the addition of large amounts of any of the vitamins studied or casein hydrolysate The growth requirements of this strain are undetermined

(4) Quantitative studies with the vitamin-deficient strains indicated that the amount of growth in the asparagine broth containing super-optimal amounts of the required vitamins did not in any case attain the value (dry weight of mycelium) obtained in a natural medium, heart infusion broth ('Difco") It was further shown that the optimum requirements for the vitamins was dependent on the medium employed

The smallest amount of inositol which would allow growth in asparagine broth ranged between 1 and 10 gamma per cc depending on the strain studied which required this vitamin The amount of growth produced was never more than 2 mg and could not be increased by further addition of inositol

The smallest amount of thiamine, in the presence of super-optimal amounts of inositol, which would allow growth in asparagine broth ranged between 0 002 and 0 004 gamma per cc, depending on the strain which required this vitamin As the thiamine was increased, a corresponding increase in amount of growth was observed Maximum growth was obtained with 0 02 gamma of thiamine per cc

The smallest amount of pyridoxine, in the presence of super optimal amounts of inositol and thiamine which allowed growth in asparagine broth of the strain which required this vitamin was 0 002 gamma per cc As the pyridoxine was increased, a corresponding increase in the amount of growth was observed Maximum growth was obtained with 0 01 gamma of pyridoxine per cc

The smallest amount of either nicotinic acid or nicotinamide which would allow the growth of *T equinum* in an ammonium chloride broth was 0 05 gamma per cc The smallest amount of l tryptophane which would allow the growth of this organism in the absence of the vitamin growth factor was 125 gamma per cc

(5) The close relationship of the faviform trichophytons to *T schoenleini* has been shown, from the early morphological studies by Sabouraud and Bodin to the present study of growth factors However, clarity in the literature and a better understanding of these organisms and the diseases which they produce may best be accomplished by distinguishing the faviform trichophytons as a group separate from *T schoenleini* This is proposed on the following basis:

(*a*) The faviform trichophytons are primarily infectious agents which cause ringworm in cattle and other domestic animals while *T schoenleini* causes a specific disease, favus, which is usually transmitted from man to man

(*b*) The faviform trichophytons (with few exceptions) produce a deep, suppurative type of lesion in man The parasitism of the hair is typically large-spored and ectothrix and the hairs are broken off close to their roots This is in contrast to the usual scutular lesions produced by *T schoenleini* and the endothrix invasion of the length of the hairs

(*c*) The faviform trichophytons are definitely stimulated by enriched or whole grain mediums with production of vigorous growth with microconidia and macroconidia *T schoenleini* strains show only slight stimulation on such mediums, and, when this occurs we have observed only few microconidia and no macroconidia

(*d*) Of twenty strains of faviform trichophytons studied, eighteen showed essential vitamin deficiencies for inositol and thiamine and in one instance for pyridoxine as well One member of the faviform group was shown to require nicotinic acid None of the six strains of *T schoenleini* studied showed an essential vitamin deficiency nor were any of the strains stimulated by large amounts of any of the vitamins studied

(*e*) One strain which we have studied *T album* (Baudet and Stuhmer), appears to be very closely related to *T schoenleini* and possibly represents an intermediate stage between the vitamin-deficient faviform trichophytons and the *T schoenleini* strains This strain showed no vitamin deficiencies, but it lacked the ability to produce some other substances present in natural products which were required for vigorous growth and production of spores

Bibliography

1 Fowle, L. P & L K Georg 1948 Suppurative ringworm contracted from cattle Arch Derm & Syph 58 780 793

9 Castellani A & A J Chalmers 1919 Manual of Tropical Medicine, 3rd ed Wood New York
10 Robbins, W J, J F Mackinnon, & R Ma 1942 Vitamin deficiencies of *Trichophyton discoides* Bull Torrey Club 69 509
11 Burkholder, P R, & D Moyer 1943 Vitamin deficiencies of fifty yeasts and molds Bull Torrey Club 70 372
12 Schopfer, W H, & S Blumer 1943 Zur Wirkstoffphysiologie von Trichophyton album Sab Ber schweiz bot Ges 53 409
13 C
14 G
15 Cazalbou, M L 1914 Contribution à l'étude des trichophytons à culture favi forme Rev gen med vet 24 1
16 Gammel, J A & J L Work 1938 Sycosis parasitica due to *Favotrichophyton album* var *singulare* Arch Derm & Syph 38 756
17 R
18 Sc
19 M
tion of nicotinic acid derivatives in humans Jour Biol Chem
23 Bonner D & G W Beadle 1946 Mutant strains of neurospora requiring nicotinamide or related compounds for growth Arch Biochem 11, 319
24 Woolley, D W 1947 Water soluble vitamins Ann Rev Biochem 16 359
25 Schopfer, W H 1943 Plants and Vitamins Chronica Botanica Co Waltham, Mass

List of Strains

I *Trichophyton faviforme* (var *album*, *discoides*, and *ochraceum*)
Strains #1-#17, which require inositol and thiamine

(a) #1-#8 Isolated from suppurative ringworm in farmers, Pennsylvania. Contact with infected cattle established in all cases
Colonies of three varieties *album*, *discoides*, and *ochraceum*

(b) #9 Isolated by Dr I Seligman, Baltimore, Maryland
Patient presented a deep, suppurative lesion
Contact with infected animals not known
Colony of the *album* variety

(c #10-#15

(d) #16 Isolated by Dr S Auchbiesiger, Quito, Equador
Cattle in this region are heavily infected with ringworm
Colony of the *album* variety

(e) #

Strain

Stra ns #19 and #20

Stock stra n Central Bureau for Fungus Cultures Holland

II Related Species

(a) S x stra ns of *T schoenleins* recently isolated from favus cases by Miss M E Hopper, New York Hospital N Y

(b) The following stock strains from the Central Bureau for Fungus Cultures Hol land

T

NEW INSIGHT GAINED IN GENERAL PATHOLOGY AND PRACTICAL MEDICINE BY THE STUDY OF SPOROTRICHOSES

By Henri Gougerot*

Professor of Dermatology and Syphilology, Medical Faculty of the University of Paris, France

The first two cases of sporotrichosis observed were that of Schenck (the fungus of which was classified by Smith in 1898 as *Sporothrix schencki*), followed by that of Hoekten and Perkins in 1900. By clinical analogy, these latter authors connected their case with a clinical observation of Brayton which was not examined bacteriologically. These three American cases had the form of an ascending, gummatous lymphangitis, and iodide therapy was apparently much less effective than in our European cases.

These American observations were unknown in Europe, despite the fact that, in 1901, Foulerton presented an excellent summary of them before the Pathological Society of London. De Beurmann and the author were the first to take up this work in France, in 1906, and make it known there. The discovery of *Sporotrichum beurmanni* rescued the sporotrichoses from oblivion, and, thanks to this research, new cases in man and in horses were published in the United States after 1909 by Burlew, by Trimble and Shaw, by Page, Frothingham, and Paige, by Mohler, by Mervins Hyde and Davis, by Duque (in Cuba) and by many others.

The first case in France observed by de Beurmann and Ramond (1903), which was caused by an organism called *Sporotrichum beurmanni* by Matruchot and Ramond in 1905, had been forgotten when, with de Beurmann, I had the good fortune to make new observations which formed the basis of my own later work. Dor, of Lyon, was the first to use the term, sporotrichosis, in 1906. However, I proved later that his *Sporotrichum dori* was a *Nocardia* and the mycologists confirmed this view. Consequently, up to the series of studies by de Beurmann and the author, three proven observations were counted, namely, that of Schenck, of Hoekten and Perkins, and that of de Beurmann and Ramond. These isolated researches had not attracted any attention or following and remained unknown. It was not until the publication of the ten reports by de Beurmann and the author (between 1906 and 1911) that the problem of sporotrichosis was opened to serious discussion and its importance in general pathology and practical medicine pointed out.

It is not the purpose, here, to repeat a review of the field, which has been published before,[1] but to evaluate as completely as possible the new insight gained by the study of sporotrichosis in the above mentioned fields.

Parasitology. General Mycology

The genus *Sporotrichum* has brought light to many questions in general mycology, though it has failed to solve some fundamental problems. Are

* Translated from the French original by Lothar Salin, former Associate Editor of The New York Academy of Sciences.

the *Sporotricha* degenerated forms of the *Ascomycetes* which have during their parasitic life with man and the lower animals, lost their higher form of reproduction? Asci have been reported in cultures of *Sporotrichum crateriforme*, but it is very uncertain whether this fungus is a *Sporotrichum* Sartory took it for an *Endomyces*, and in all the thousands of different *Sporotrichum* cultures which I have handled, I have failed to find any asci, despite all efforts For this reason, we shall not discuss whether these organisms are degenerated fungi or whether they belong to the lower fungi and have never had a higher form of reproduction

The classification of the genus Sporotrichum is still disputed Vuillemin,[1] of Nancy, put some in the class *Conidiospora*, in the order *Sporotricha* with two principal genera, *Sporotrichum* and *Rhinocladium* He calls *Rhinocladium* the *Sporothrices* of both Schenck and de Beurmann This change of name was, however, not recognized by Matruchot who maintains the classification in the genus *Sporotrichum*[2]

To begin with, Matruchot remarks, 'The differences mentioned, such as coloring of the filaments and spores, the mode of attachment of the spores (pedicellate or sessile), are not so remarkable as the diagnoses given in the books suggest These divers characteristics are so little specialized that they can all be found on the same parasite The facts presented to us by nature cannot be put into compartments so easily '

[illegible]

specifically notes pedicellate spores Matruchot also cites other examples taken from his own work, such as *Ctenomyces serratus, etc* Thus, he thinks that the presence of pedicellate spores, which according to Vuillemin, would be a characteristic of *Rhinocladium* and distinguish it from *Sporotrichum*, can apply to *Sporotrichum* as well and consequently is of no differentiating value

[illegible]

extreme end of the last ramifications of the lateral and terminal spores, which are placed on toothlike projections The spores are globular or oval shaped and blackish " However, says Matruchot, in *Sporotrichum beurmanni*, 'the fertile filaments are not blackish they are not branched dichotomously when mature, the spores are not placed exclusively near the extreme end of the terminal filaments but, rather, on the entire wall of the fertilized branches up to a very great distance from the extreme end [illegible] ections, they [illegible] spores have [illegible] diagnosis of *Rhinocladium*, thus, cannot apply to our parasite, which has to retain the name of *Sporotrichum*—"the natural affinities of *Sporotrichum beurmanni* are elsewhere than in the genus *Rhinocladium* "

We cannot but compliment Professor Matruchot on his argument Should we, however, distinguish with him between *Sporotrichum schencki* and *beurmanni* (*loc cit*), or should they be considered as one species as was done by Verdin and Buillemin? The last named author believes that the cultures of *schencki* which were sent to us in Paris and which we studied for a long time, are actually degenerate and pleomorphic, hence their apparent difference as compared to the *beurmanni* However, at the end of his discussion, Vuillemin gives the name of *Rhinocladium beurmanni* to the parasites of both Schenck and de Beurmann, stating that "usage has sanctioned the name of *Rhinocladium beurmanni*" Other authors, nevertheless, in making the synthesis, prefer the double name *Sporotrichum schencki beurmanni*, and, in 1910, I proposed the following table

Sporotrichum (ancestral)	1st Group *Sporotrichum schencki beurmanni* common source of	*Sporotrichum schencki*	(I)
		Sporotrichum beurmanni	(II)
		and its varieties	
		Sporotrichum beurmanni var *asteroides*	(III)
		Sporotrichum beurmanni var *indicum*	(IV)
		Sporotrichum jeanselmi	(V)
	2nd Group *Sporotrichum gougeroti* etc		

Vuillemin Grigorakis, and also Dodge[4] made an attempt to change the name of *Sporotrichum gougeroti*, which was established by Matruchot, to *Dematium gougeroti* Saccardo considers this organism related to *Torula*

to this change of name for two principal reasons, namely (1) the parasite in question has colored spores, or conidia, (2) the genus *Dematium* is badly defined since "all the Dematiacea can be incorporated in it as long as only the yeast forms are considered"

Dodge also refused to accept the classification of several *Sporotricha* Thus, he makes both *Sporotrichum grecorus*, described by Mackinnon, and ... From *Sporo-*
sbyi,
iders
Sporotrichum

trichum bronchiale (Montagne), and *Rhinocladium parvulum* (Redaelli)

Guegen asks whether it would not be worth while to create a new genus *Sporotrichopsis* To quote from his argument, 'The genus *Sporotrichum* is so badly defined or rather so little defined, that a radical measure seems indicated This would consist of treating the genus *Sporotrichum* as an abandoned cemetery where no one is buried any longer By making *Sporo*

somebody has taken the trouble to study their structure The presence of "sympodially born conidia," which has not been noted in the genus *Sporotrichum*, constitutes, according to Guegen another argument in favor of the creation of a new genus

The forms in the tissues are different from those in vitro cultures The writer believes that he was the first to describe the short forms and to have shown that they are not spores as other authors thought These forms are degenerated, due to adaptation in the struggle for existence in the tissues The writer has also drawn attention to the budding yeast form, similar to that encountered in the blastomycoses

A thorough study of the yeast forms of the sporotrichoses has been made by Charlotte C Campbell, who showed that this phase persists in Francis's solution (glucose agar with blood with cystine added) as long as this is incubated at 37°C On the other hand transplanted on Sabouraud's agar, a yeast form *Sporotrichum* will exhibit the typical filamentous form within 48 hours at room temperature

Splendoré, of São Paulo, has described star like forms in *Sporotrichum asteroides*, which the author with Dodge believes to be a simple variety of *schenckii-beurmanni* Spillman and Gruger in animals and Greco in man, have noted radiating actinomycosiform specimens whose presence in the tissues was also pointed out by Moore and Ackerman Widal and Abrami discovered the seroagglutinations complement fixations and group reactions This method is useful not only to diagnose sporotrichosis, but it can also, owing to the shortness of the reactions be used to diagnose other mycoses, particularly actinomycoses However I have shown several causes of error here, and likewise in the intradermal reactions with sporotrichin (killed fungus antigen) For example a simple saprophyte of *Sporotrichum*, in the bucco-pharynx, or even that of a different fungus perhaps a yeast form in the digestive tube can, by group reaction, give seroagglutinations of up to 1/150, with medium complement fixations This would give the erroneous impression that a lesion was of sporotrichotic nature, when actually it is due to an entirely different agent

of the *Sporotricha*, namely, soluble toxins insoluble toxins (endotoxins), etherosporotrichosine, chlorosporotrichosine, *etc* We also analyzed their fermentative power on various substances, comparing the different species and stressing their instability in this respect While these criteria are use-

ful in bacteriology, they have thus become of rather questionable value in mycology

De Beurmann, Ravaut, Verdin, and the author have studied sensitizations and group sensitizations, above all the intradermal reactions, and have opened the chapter of polymycoses This research has led us to realize that the sporotrichoses may take on an appearance similar to that of bacterial infections

Practical Medicine

From the point of view of practical diagnosis, I have popularized Sabouraud's technique of culture at room temperature on French proof agar, showing that incubated cultures give slower and less constant results (and, above all, less characteristic colonies) Thanks to this technique, the mycological diagnosis of sporotrichoses has become simple and rapid, and has come within the reach of even the most isolated practitioner, since this *method of culturing makes it possible to dispense with an incubator*, and since, moreover, the macroscopical aspect of the colonies is sufficiently characteristic without necessitating laboratory examination

In order to speed up bacteriological diagnosis, I have drawn attention to "the run of pus on dry glass" The originating colonies are easy to dis- [illegible] preparation or stain [illegible] gi, I have [illegible], I showed [illegible] with scar formation This can be done not only by using the serum diagnosis of Widal and Abrami (which lacks specificity), but above all through culture of the bucco-pharynx, since the fungus frequently remains saprophytic on the mucosae

Frequency of Occurrence *The frequency of mycoses depends largely* on the thorough and systematic search for them In March, 1907, I had isolated four cases, by the end of 1907, thirty cases, by the end of 1908, sixty, and finally, by the end of 1910, more than two hundred Observations have been reported from all French provinces, from all countries on the five continents, and in patients of all ages Consequently, physicians in any country may come across sporotrichosis The apparent rarity of the disease is due to the fact that it has become so classical that only observations which present interesting new details are reported Nevertheless, [illegible] observations, [illegible] pearance of [illegible] d by Watrin

All the same, the sporotrichoses have certainly decreased in frequency, at least in France, despite our systematic search for them This seems also to apply to other continents, for example, R O Noojin and J L Callaway mention that they have found only seven cases in ten years The question can be asked whether this is due to the extinction of all human and animal reservoirs by improved diagnosis and treatment with iodides Infection

from human to animal and *vice versa*, is, of course, exceptional at best In any case, there is no question of contamination by improperly sterilized syringes (certain specialists have advanced this bizarre theory), since in our well-conducted investigations we have never seen an "epidemic" and our patients never received any injections

Clinical Polymorphism In medical practice, my own observations, supplemented by those of other authors have described numerous forms ranging from a gumma and even large abscess to acne and sporotrichotic pityriasis

Lesions may occur in all tissues In the bones, joints and synovia, sporotrichoses exhibit everything that can possibly be imagined from periostitis to abscesses in the interior of the bone spina ventosa and spontaneous fractures, from simple hydarthrosis to white swelling as well as various forms of synovitis I have stressed the singular frequency of lesions in the bones which is about 10 per cent in disseminated sporotrichoses—a number not reached by syphilis

In the viscera, cases of orchi-epididymitis have already been reported and also pulmonary congestion with hemoptysis[9] and pyelonephritis (Rochard, Duval, and Bodoleo) There has been a recent report by Andre Martin of a renal sporotrichosis simulating the appearance of a sarcoma later that of tuberculosis[10] Further reports have been made of a febrile form (de Brissaud and Rathéry) and a fortunately exceptional mortal one (de Roux Lacroix, Banks) Sporotrichoses can simulate the appearance of blastomycoses (H G Adamson) actinomycoses (Gougerot), diphtheria (Banks), *etc* As I have repeatedly stated The sporotrichoses for all these reasons, are of interest not only to the dermatologist, but also to the internist, the surgeon, the oculist obstetrician pediatrician and psychiatrist, as well as in forensic medicine[11] It is necessary to make a diagnosis immediately without waiting until the patient becomes cachectic because at that stage, death is frequently inevitable despite treatment with iodide The prognosis, thus, truly depends on the scientific reliability of the examining physician with the exception of certain rare cases with associated tuberculosis where the iodide treatment is impossible

In practice, I have described and stressed several diagnostic phenomena, namely, the gummatous form which reappears upon incision and again reappears under the scar of the incision, disclosing the typical dome shaped form with softening in the center, the polymorphism of the ulcerations (tubercular, syphilitic, or other in appearance) which may be evident either on different ulcers situated closely together or on different sectors of the same ulcer, and the rapid relapse after premature cessation of the iodide treatment

As regards general pathology, this group of observations shows that, as in bacterial infections in particular those of tuberculosis and syphilis one single virus can exhibit very different clinical forms and that the same clinical phenomenon as for example the gumma may be due to different viruses or fungi

Histology The histological formula of the *Sporotricha* can be established

as consisting of three zones, namely, (1) the central zone formed of polynuclear cells and macrophages simulating the presence of a pyodermia, (2) median zone, tubercular in appearance, with epithelioid cells, giant cells, and tuberculoid follicles, and (3) the external zone, composed of lymphocytes and connective tissue cells, basophilic, with comparatively intense vascularity, frequently exhibiting almost a syphilitic sclerosis These three reactions may even coexist without any order, *i e*, they may be mixed up particularly in the verrucous and vegetating sporotrichoses

This histological reaction is nonspecific, since it is also encountered in other mycoses and even in the foreign body reaction of tissues, as my clinical observations have shown In this connection, the experimental study by Vaucher and myself, especially with pepper, should be noted

The histogenesis of the lesions is much less rapid than that of syphilis and tuberculosis, owing to the manner of the sporotrichotic process Thus, we have been able to show, with a series of excised tissues, how the vascular stage is transformed into giant cells and, subsequently, into tuberculoid follicles and gummatous tissue with the three zones This confirms the opinion of Gernil and other French authors who believe that the formation

that the sporo-
xhibit *pyococcic*,

Etiology and Pathogenesis Through my clinical observations and experimental studies (particularly in collaboration with Vaucher), the etiology and pathogenesis of the sporotrichoses have been clarified inasmuch as they exhibit all possible clinical forms and are capable even of taking the shape of a bacterial infection Their reservoir lies within the limits of nature (as for the blastomycoses, actinomycoses, *etc*) I have demonstrated this by finding "wild" *Sporotrichum beurmanni* on an oat plant, a horsetail and a small beech tree, in the French Alps in 1908 Upon cultivation these wild specimens were not virulent, only after passing from rat to rat did they become pathogenic Soon afterwards, Sartory[12] found the same *Sporotrichum* on an ear of wheat, the first generation was non pathogenic, but, after passage through several animals, the organism became virulent

Animals are but rarely the source of contagion, and they themselves are generally infected by plants Pertinent observations here have been recorded by Lutz and Splendoré, as well as by Jeanselme and Paul Chevalier (rat sporotrichosis transmitted to man), Page, Frothingham, and Paige, also Mervins Hyde and Davis, and Mohler (horse sporotrichosis) R L Sutton (human contamination), T C Jones and Fred D Maures (mule sporotrichosis—see especially Carougeau's case of contamination of the veterinarian treating an affected mule), Gougerot and Caraven (dog sporotrichosis), Wyss Lauzin (parrot sporotrichosis, and transmission to the human by a bite), and Olson (rodent sporotrichosis, with transmission to man)

The same phenomena are found here as in tuberculosis, such as primary chancre at the site of infection (see my observation No 12) or lymphangitis

without visible portal of entrance (No 13) saprophytism on the mucous membranes and germ carriers (J Thiry) infection through the intestinal canal and dissemination through the lymphatics or the blood circulation (Widal and Weill) The same factor of lowered resistance accompanied by progressive adaptation and virulence as in diabetes and tuberculosis has been demonstrated on the sporotricha found in nature Increasing sensitization in man has been elicited by the intradermal reactions reported by Paul Ravaut Verdin and myself and thus the importance of the human reservoir has been stressed Also hybrid infections such as combinations of sporotrichosis and tuberculosis have been encountered (Achard and Louis Ramond personal observations

Experimental Reproduction As outlined in the foregoing our long experimental studies have clarified the clinical treatment the histology etiology and pathogenesis of sporotrichosis However experimentation has taken the lead from clinical studies as regards the visceral forms which give a picture of the bone infections and other forms With Vaucher I have even outlined a possible hereditary sporotrichosis thus again demonstrating the similarity to bacterial infections and syphilis

Treatment The iodide treatment has been patiently evolved and regulated according to the four well known categories of patients (1) tolerant to iodide, (2) partially intolerant to iodide (3) completely intolerant to iodide, and (4) local lesions not responsive to iodide I have stressed the following rules for this treatment doses of 4 gm daily necessity for supervising the tolerance of iodide which may give a clue to possible latent tuberculosis necessity of prolonging treatment at least one month beyond the apparent healing of the lesions since relapses frequently occur otherwise consolidating treatment, observation of a possible pharyngeal reservoir of parasites, and recognition of the gravity of cases associated with tuberculosis

I have made studies parallel to the work of Achard and Louis Ramond proving that the iodide treatment is not effective through direct antiseptic action since it is possible to cultivate the sporotrichosis fungi *in vitro* in from moderately to very strong iodide solutions Rather the iodide acts through stimulation of the macrophages and of the defense mechanism of the tissues

Recent research particularly in the United States has shown decided effects of the sulfonamides R O Noojin and J L Callaway for example used sulfonamide and sodium salt of sulfapyridine in local treatment with a 5 per cent oily suspension

Conclusion

Through all the studies enumerated in this paper the sporotrichoses have been classified pathologically among the infections *i e* they have been separated from other mycoses The study of mycoses has thus been given new vigor and it is no longer necessary to contrast them against bacterial infections as has been done in the past but rather connections between the two can be established

The study of the sporotrichoses, through systematic research in all the mycoses, has led to the discovery of several new forms as, for example, hemisporosis, caused by *Hemispora stellata* (Gougerot and Caravell), oïdiomycosis or mycodermosis, caused by *Oidium* or *Mycoderma cutaneum* (Gougerot, de Beurmann, and Vaucher), nocardiosis of Carougeau, gummatous parendomycosis, caused by *Parendomyces balzeri*, and mycodermosis caused by *Mycoderma pulmoneum* (Gougerot, Balzer, and Burnier). In collaboration with Duché,[13] I found disseminated polyepidermomycosis of the intertriginous regions (leaving their basis intact), with the presence of *Epidermophyton inguinale*, *Malassezia furfur*, and a new fungus, *Aspergillus endoticі*, also, a new subcutaneous mycosis, caused by *Debaromyces klockeri*. Finally, with Burnier and Duché, I discovered a vegetating and ulcerative mycosis, caused by *Cephalosporium griseum*.

The classical clinical analysis of the sporotrichoses was made by my old teacher, Louis Landouzy,[14] who stressed the practical prognostic, therapeutic, and economic importance of the sporotrichoses. Time has indeed passed since another of my great teachers accused me of being a youthful hothead "trespassing on the sacred ground of syphilis and tuberculosis." I have repeatedly drawn attention to the faulty diagnosis of tuberculosis, syphilis, chronic osteomyelitis, glanders, and even cancer, which has led to amputation and death, and I can but once again repeat the words of Professor Grasset, the famous neurologist: "A physician who is not familiar with the latest research on sporotrichosis runs the risk of letting patients die who could have been saved by the judicious administration of potassium iodide."

Bibliography

[illegible] 1912. La Nouvelle Pathologie Dermatologique,

[illegible] 10 in the series

[illegible] (3) 253–264

[illegible] & Syph 5 27

[illegible] July

[illegible] accidents and at work. Soc

[illegible] 1936 Occupational sporo-

[illegible] 527–634

GROWTH REQUIREMENTS OF DERMATOPHYTES

By William J Robbi

Department of Botany Columbia University and Direct . . . nical Garden

It is not my intention to discuss the growth r . . . e dermato-phytes in general, but to confine my attention . . . th which we have been mainly concerned One of these . . . *coides* the other is *Trichophyton mentagrophytes*

The strain of *T discoides* with which we . . . ed from Dr Juan Mackinnon of the Institute of Hygiene M . . . uay It was found that for growth this isolation requ r . . . of molecular . . . to grow on a . . . paragine The . . . medium permit- . . . re necessary

Little or no improvement in growth was . . . with the addition of any one of the three or any two of the three

The thiamine deficiency could not be satisfied by the pyrimidine component of thiamine, by thiazole, or by a mixture of the two thiamine intermediates Neither calcium phytate nor a phosphatide containing inositol obtained from Dr D W Woolley were as satisfactory as inositol The phosphatide substituted for inositol more nearly than did the calcium phytate

The minimum effective quantities of the three vitamins were not determined, but the addition of 0 01 mg of inositol to 8 ml of medium which contained both thiamine and pyridoxine produced a marked effect on growth The maximal effect was obtained with between 0 1 and 0.5 mg of inositol The addition of 0 001 mμ mole of pyridoxine to 8 ml of medium

. . .

moles of thiamine

Neopeptone in the amounts we used did not contain sufficient thiamine for maximum growth Better growth was obtained when the mineral-dextrose peptone medium was supplemented with thiamine Inositol or a physiological equivalent material was found to be present in hydrolyzed gelatin, hydrolyzed casein, hydrolyzed egg albumen, and peptone, as evidenced by the growth of the organism on these media supplemented with thiamine and pyridoxine but no inositol

Although it was possible to obtain considerable growth on the basal medium of mineral salts dextrose, and asparagine supplemented with thiamine, pyridoxine, and inositol, better growth was obtained on a thiamine peptone medium or on gelatin hydrolysate or casein hydrolysate supplemented with the necessary vitamins It appeared, therefore, that gelatin

hydrolysate, casein hydrolysate, and peptone contained unidentified factors important in the growth of *T discoides* Although we were not successful in duplicating the effect of the protein hydrolysates by the substitution for them of a mixture of amino acids and purine bases, it is probable that the action of the hydrolysates was an amino acid effect

Our investigations, therefore, showed that the strain of *T discoides* which we used evidenced complete deficiencies for molecular thiamine, pyridoxine, and inositol and partial deficiencies for unidentified growth substances present in peptone and hydrolysates of gelatin or egg albumen The unidentified factors are probably amino acids

salts, dextrose, and asparagine Its growth is markedly improved by the addition to the basal medium of peptone, or hydrolysates of casein, egg albumen, or gelatin

In contrast to *T discoides*, the isolation of this organism has no vitamin deficiencies No improvement in growth has been observed from the addition of vitamins to the basal medium or to the basal medium supplemented with vitamin free protein hydrolysates

Since this fungus evidenced no deficiencies for any of the known vitamins and its growth was markedly improved by hydrolysates of egg albumen or highly purified gelatin, and of vitamin free casein, it seemed probable that the active substances were amino acids It was found that the organism would grow on a basal medium containing asparagine or any one of the following 14 amino acids glycine, dl alanine, dl valine, l leucine, d isoleucine, dl phenylalanine, d glutamic acid, dl aspartic acid, dl serine, l-cystine HCL, l tyrosine, *l* proline, d arginine HCL, and *l* histidine *HCL* Only 5 of 19 amino acids tested gave little or no improvement in growth These were tryptophane, threonine, hydroxyproline, methionine, and lysine Ammonium nitrate was nearly or completely unavailable However, we were not able to duplicate completely the effects of casein hydrolysate, for example, by any mixture of amino acids we prepared

Our strain of *T mentagrophytes* was able to use asparagine or any one of 14 amino acids as a source of nitrogen, but was nearly or completely unable to utilize ammonia or nitrate nitrogen It appeared able, therefore, to

able amino acid in the sense that the fungus failed to grow unless a particular amino acid was *furnished in the nutrient medium* Although *T mentagrophytes* is capable of making all the amino acids needed for its proteins from a single amino acid or from asparagine, a mixture of certain amino acids was superior to an equal amount of any single one This we believe, are incorporated into the metabolic trans- In other words the

mechanism for nitrogen transformation in this organism is deficient It lacks, nearly or completely, the machinery necessary for utilizing inorganic nitrogen Also, its mechanism for transforming asparagine or a single amino acid into those required for the construction of cell substance works slowly

One of the complicating factors in the study of the nutrition of the dermatophytes is the freedom with which many of them produce pleomorphic forms which may differ in their physiology from the organism from which they are derived Pleomorphic forms have long been recognized in this group They are spontaneous irreversible mutations

We have investigated chiefly the pleomorphic forms of T mentagrophytes These mutants develop as cultures of this organism age Portions of the mycelium change in growth habit Many pleomorphic types can be isolated which differ in rate of growth, degree of sterility morphological character of the colony, pigment production, or some physiological character All cultures, if sufficiently old, are characterized however by a vigorous, white, more or less fluffy, almost sterile form The growth of the most vigorous forms is many times that of the original isolate In a liquid medium containing asparagine as a nitrogen source the normal form produces three or four milligrams of dry matter in the course of three weeks A pleomorphic form, in the same time in the same medium may form a hundred milligrams or more

We have found it possible to maintain the slow growing freely sporulating form, which we call the normal or N form, by making transfers to fresh media at weekly intervals By such a procedure, we have kept *T mentagrophytes* for more than four years, apparently unchanged The pleomorphic forms, too, can be isolated, grown in pure culture and maintained if they are transferred at weekly intervals

The spontaneous change from normal to pleomorphic occasionally appears in cultures incubated at 35° C as early as eight days after inoculation though two or three weeks are usually required and within four or five weeks at most all cultures will have become pleomorphic It is not therefore, a hit-or miss process Merely by allowing the cultures to age, all of them will change from a slow growing, freely sporulating form to one which grows rapidly and is nearly or completely sterile

From the standpoint of this discussion, these pleomorphic strains are important because they differ in their growth requirements from the original form from which they are derived For example some of the rapidly growing mutants of *T mentagrophytes* are able to use inorganic nitrogen, which is nearly or completely unavailable to the normal form (N) from which they arose The pleomorphic forms are characterized by a greater ability to transform ammonium salts and also asparagine into those amino acids necessary for the construction of their protoplasmic proteins These transformations are probably enzymatic, which means that in becoming pleomorphic the fungus has developed new or more effective enzyme systems than exist in the normal form

I do not mean that the only difference between the normal and pleo-

hydrolysate casein hydrolysate, and peptone contained unidentified factors important in the growth of *T discoides* Although we were not successful in duplicating the effect of the protein hydrolysates by the substitution for them of a mixture of amino acids and purine bases, it is probable that the action of the hydrolysates was an amino acid effect

Our investigations, therefore, showed that the strain of *T discoides* which we used evidenced complete deficiencies for molecular thiamine, pyridoxine, and inositol and partial deficiencies for unidentified growth substances present in peptone and hydrolysates of gelatin or egg albumen The unidentified factors are probably amino acids

The strain of *T mentagrophytes* that we investigated was isolated from

addition to the basal medium of peptone, or hydrolysates of casein, egg albumen, or gelatin

In contrast to *T discoides*, the isolation of this organism has no vitamin deficiencies No improvement in growth has been observed from the addition of vitamins to the basal medium or to the basal medium supplemented with vitamin free protein hydrolysates

s found that the organ

paragine or any one of

valine, l leucine d iso-

leucine, dl phenylalanine, d glutamic acid, dl aspartic acid, dl serine, l-cys tine HCL, l tyrosine, l proline, d arginine HCL and l histidine HCL. Only 5 of 19 amino acids tested gave little or no improvement in growth These were tryptophane, threonine, hydroxyproline, methionine, and lysine Ammonium nitrate was nearly or completely unavailable However, we were not able to duplicate completely the effects of casein *hydrolysate, for* example, by any mixture of amino acids we prepared

Our strain of *T mentagrophytes* was able to use asparagine or any one of 14 amino acids as a source of nitrogen, but was nearly or completely unable to utilize ammonia or nitrate nitrogen It appeared able, therefore to transform asparagine or any one of the several amino acids without previous ammonification into all the various amino acids required for the construction of its protoplasmic proteins We found no evidence for any indispens

unless a particu-

lthough *T menta*

d for its proteins

of certain amino

This we believe

orporated into

etabolic trans

ner words the

mechanism for nitrogen transformation in this organism is deficient It lacks, nearly or completely, the machinery necessary for utilizing inorganic nitrogen Also, its mechanism for transforming asparagine or a single amino acid into those required for the construction of cell substance works slowly

One of the complicating factors in the study of the nutrition of the dermatophytes is the freedom with which many of them produce pleomorphic forms which may differ in their physiology from the organism from which they are derived Pleomorphic forms have long been recognized in this group They are spontaneous irreversible mutations

We have investigated chiefly the pleomorphic forms of *T mentagrophytes* These mutants develop as cultures of this organism age Portions of the mycelium change in growth habit Many pleomorphic types can be isolated which differ in rate of growth, degree of sterility morphological character of the colony, pigment production, or some physiological character All cultures, if sufficiently old, are characterized however by a vigorous, white, more or less fluffy, almost sterile form The growth of the most vigorous forms is many times that of the original isolate In a liquid me

hundred milligrams or more

We have found it possible to maintain the slow growing freely sporulating form, which we call the normal or N form, by making transfers to fresh media at weekly intervals By such a procedure, we have kept *T mentagrophytes* for more than four years, apparently unchanged The pleomorphic forms, too, can be isolated, grown in pure culture and maintained if they are transferred at weekly intervals

The spontaneous change from normal to pleomorphic occasionally appears in cultures incubated at 35° C as early as eight days after inoculation, though two or three weeks are usually required and within four or five weeks at most all cultures will have become pleomorphic It is not, there

From the standpoint of this discussion, these pleomorphic strains are important because they differ in their growth requirements from the original form from which they are derived For example, some of the rapidly growing mutants of *T mentagrophytes* are able to use inorganic nitrogen which is nearly or completely unavailable to the normal form (N) from which they arose The pleomorphic forms are characterized by a greater ability to transform ammonium salts and also asparagine into those amino acids necessary for the construction of their protoplasmic proteins These transformations are probably enzymatic which means that in becoming pleomorphic the fungus has developed new or more effective enzyme systems than exist in the normal form

I do not mean that the only difference between the normal and pleo-

morph c stra ns s a change n the ab l t of the org n sm to ran f rm a

of a dermatophyte which has a specific vitamin deficiency might be able to synthesize the vitamin which the normal form is unable to make We have found this to occur in pleomorphic forms (sectorial mutants) of *Fusarium avanaceum* The original isolation lacked the power to synthesize biotin but a vegetative mutant developing spontaneously from the original isolation was able to make its own biotin

One of the peculiarities of the dermatophytes is their relation to hydroxyproline This amino acid a common constituent of proteins was found to

Rhodotorula

The growth of *T mentagrophytes* on a basal medium containing mineral salts dextrose and asparagine was almost completely inhibited by one part of l hydroxyprol ne in 20 000 parts of medium and a reduction in growth was observed with one part in 50 000 parts of medium Two pleomorphic forms of *T mentagrophytes* proved to be more resistant to hydroxyproline One of them required one part of hydroxyproline in 1600 parts of medium and the other one part in 4000 for complete or nearly complete

parts of medium

Trichophyton purpureum Microsporum canis Epidermophyton floccosum and a granular form of *Trichophyton gypseum* were also susceptible to the injurious effects of hydroxyproline The *Ep dermophyton* was the most sensitive Almost complete inhibition was obtained on the addit on of one part of hydroxyproline to 40 000 parts of the basal medium *Microsporum canis* was the most resistant The growth of this fungus was reduced but not prevented by one part of the hydroxyproline in 1600 parts of medium

In contrast to the effect of this amino acid on the dermatophytes was its action on such organisms as *Penicillium notatum Rhi opus nigricans* and *Fomes pini* These fungi showed no reduction in growth even in the presence of one part of hydroxyproline in 1600 parts of medium

The injurious effect of hydroxyproline on the dermatophytes was less pronounced in med a containing peptone or casein hydrolysate than in

ne The effect of each of 14 am no acids on the normal form of at least in part o be therefore

some evidence for an antagonism between l prol e and hydroxyprol ne

One of the curious responses to hydroxyproline was observed with *T purpureum* The growth of this organism was inhibited almost completely

by one part of hydroxyproline in 1600 parts of medium Also, a marked reduction in growth was obtained with one part of the amino acid in 4000 parts of medium With small amounts, one part in 40 000 or one part in 20 000, growth was stimulated The colonies obtained were larger than observed on the basal medium

The effects of hydroxyproline on the dermatophytes is interesting because it seems to be evidenced on these organisms as a group, which suggests a common physiological characteristic It is also of interest because proline is an available source of nitrogen for these organisms and the only difference between proline and hydroxyproline is a hydroxyl radical in the fourth position on the molecule

latter, which are the more resistant, are also better able to supply their nitrogen requirements from inorganic nitrogen or from a single source of organic nitrogen

This has been only a fragmentary presentation of the growth requirements of dermatophytes There has been no discussion of their relation to hydrogen ion concentration, to reduced sulphur, or to carbon sources or many other of the fundamental nutritional factors which should be considered There has been no attempt to review here the contributions made by others to this subject The paper has been limited to the specific researches with which the author and his associates, Dr Roberta Ma and Dr Ilda McVeigh, have been concerned

References

ROBBINS W J, J E MACKINNON, & R MA 1942 Vitamin deficiencies of *Trichophyton discoides* Bull Torrey Bot Club 69 509–521

ROBBINS W J & R MA 1945 Growth factors for *Trichophyton mentagrophytes* Am Jour Bot. 32 509–532

ROBBINS, W J & I McVEIGH 1946 Effect of hydroxyproline on *Trichophyton mentagrophytes* and other fungi Am Jour Bot 33 638–647

ROBBINS W J 1947 Some basic contributions to the cancer problem from the study of plants Ann N Y Acad Sci 49 75–86

FUNGUS ANTIGENS AND THEIR IMPORTANCE AS SENSITIZERS IN THE GENERAL POPULATION

By Samuel M Peck

Mount Sinai Hospital New York N Y

Disease manifestations caused by both bacteria and fungi can be divided into those which are directly due to these organisms and those special forms which have arisen because of the development of sensitization to the organisms and/or their products The latter have been grouped under the general heading of cutaneous microbids[1]

The clinical manifestations of microbids depend on a development of an acquired hypersensitivity to the organisms and/or their products after the primary infection has existed for some time The degree of acquired hypersensitivity is dependent on the causative organism, on individual predisposition and on many other factors which cause more intimate contact between the living organisms and the living cells

In the group of microbids, we have trichophytids when the trichophyton fungus is the primary cause of the lesion, epidermophytids when an epider

ture has often been shortened to "ids "

The allergic manifestations due to fungi which are most commonly encountered in the general population are those associated with the superficial fungus infections Of these, almost all are associated with infection due to *T mentagrophytes* especially dermatophytosis of the feet

Pathogenesis of Trichophytids

The *Epidermophyton* and *Trichophyton* fungus grow in the nonliving layers of the skin and its appendages Because of this fact, they give rise to clinical manifestations which are primarily of a very superficial nature Marked inflammation does not develop unless living structures are invaded Invasion by the fungi of living structures or contact of fungi or their products with the living parts of the skin probably initiate the hypersensitivity with its resulting allergic manifestations W Jadassohn and Sulzberger have shown in experimental fungus infections in guinea

body but fungus infection developed only in the nonliving parts of the skin When the animal was killed shortly after injection however, and the internal organs were cultured fungi readily grew from this dead tissue It is of interest to note that the lens of the eye (Jadassohn Rehsteiner) could be infected with fungi just like the keratin layers of the skin

A *sine qua non* for the development of trichophytids is a hypersensitivity

to fungi or their products This sensitivity is revealed by a positive tricho-
phytin reaction Fortunately the trichophytin test represents a group
reaction in the majority of instances This means that the test does not
usually have to be made with the identical fungus causing the lesions
Previously it was thought to be present only in the deep inflammatory fungus
infections such as kerion celsi Recently however, it has been demon-
strated that even superficial fungus diseases were frequently accompanied
by sensitivity to trichophytin The hypersensitivity to fungi develops
after the primary infection has existed for some time The period between
onset of infection and the development of hypersensitivity varies from a
few months to several years depending on the type of fungus the site of
the primary infection and the infected individual It is very difficult to
determine whether atopy plays a role in the development of this hypersensi-
tivity In the available statistical data a history of atopy seems rather un-
important especially as far as the epidermophytids on the hands are con-
cerned

Trauma, such as treatment with strong ointments X rays or continu-
ous maceration which is frequently seen between the toes forces the fungi
or perhaps their products into the blood stream These in turn com-
ing in contact with the hypersensitive skin give rise to trichophytids
The localization of the trichophytids may depend on the anatomy of the
circulation or other rather obscure moments The work of Truffi with
fungi and of W Jadassohn with tuberculosis has shown that localization
can be determined experimentally by causing rupture of capillaries thus
allowing the circulating noxa to become free and reach the skin The
circulating organisms are changed by immune bodies in the blood and
reach the skin in an attenuated state To reach the keratin layer (i e
the dead tissue in which they can grow) they pass through the living struc-
tures which have become hypersensitive The resulting interaction destroys
the majority of them and gives rise to the trichophytids If any organ-
isms pass through these barriers and reach the dead tissues they can be
demonstrated in the trichophytids This is rather a rarity

If we were to assume that not only the fungi but also their toxins can
give rise to trichophytids it would be easy to understand why trichophytids
are usually sterile Such an assumption however would not explain the
localization of epidermophytids on the hands only secondary to the fungus
infection of the feet in the presence of a generalized skin sensitivity Fur-
thermore it has not been possible to demonstrate such circulating toxins
while positive blood cultures for fungi identical with those causing the pri-
mary infection have been obtained

Williams has pointed out that once hyperse
lished even the primary lesion is
epidermophytids This explains th
demonstrating fungi from such are
trichophytids will give negative microscopic results ... us that scrapings from

It is within the realm of theoretical possibilities that contact with pri-
mary lesions can cause transportation of the organisms to other parts of

humans This was the first time that a spontaneous experimental trichophytid had been reproduced in a human subject

While the dyshydrotic trichophytids and the type known as dyshydrosis *Lamellosa sicca* are fairly easy to diagnose, the eczematoid trichophytids, especially when they occur on the hands, are often impossible to differentiate from ordinary eczema It is just in this group that failures of diagnosis occur, because of the great prevalence of epidermophytosis of the feet with its accompanying positive trichophytin reaction This difficulty of diagnosis and the need for methods of differentiation is well recognized by all workers in the field It is for this reason that serological criteria for diagnosis have been attempted

Recent literature, both foreign and American, has become increasingly filled with the discussion of levurids those mycids which are due to monilia Ravaut, a pupil of Sabouraud, described the first cases Very interesting examples of such eruptions have been demonstrated by Rarrel, Hopkins and others As Bloch has pointed out, in discussing the research of Staehelin from his Institute, it is very difficult to prove that an eruption is a

oidiomycin is based on true hypersensitivity because of its practically one hundred per cent incidence in the adult The last, however cannot be considered as very strong negative evidence when we consider that the number of the oidiomycin reactions increases with the age group and that, *in certain classes of the population, even the tuberculin reaction may* approach one hundred per cent incidence However, one of the most important steps in the evidence necessary to prove the existence of levurids, namely, positive blood cultures taken under rigid control conditions, is lacking

tive oidiomycin reaction

Incidence of Dermatophytosis in the General Population

When the incidence of dermatophytosis in the general population was discussed, until the recent epidemic of tinea capitis, the discussion was mainly concerned with fungus infections of the feet, hands and groins A number of authors have investigated this problem The largest group of cases included in one study was that carried out by the Public Health Service in 1943-1944[2] Approximately 2100 people living in the District of Columbia, New Jersey, Connecticut, Indiana, and Louisiana were included in the pH survey *The effects of different seasons on* the incidence of dermatophytosis also were studied The fungus infections encountered rarely affected parts of the body other than the hands and feet In that study, 1393 men and 733 women were examined Their ages varied from 17 to 70 years, but most of those examined were in the second and *third*

decade Based on clinical and cultural grounds the classification of the examinees were as follows positive 590 (27 79 per cent), doubtful 714

group 214 (29.31 per cent) were in the doubtful group and 317 (43 42 per cent) were in the negative group

It was shown by cultural study that some patients in the clinically doubtful group had true dermatophytosis There was no significant difference in sex incidence When the same personnel were examined at different seasons, the number of clinically negative patients rose from 13 per cent to 19 per cent in the summer to 54 per cent in the winter Thus it can be seen that there is a definite influence of seasons as far as clinical evidence of the activity of fungus infections is concerned The most frequent pathogenic fungus recovered was *T gypseum* *T purpureum* was next in frequency and *E inguinale* was only recovered from an occasional case All of the cultures of *T purpureum* were recovered in one locality

The work of Osborn and Hitchcock[3] seems to show that the women are less affected with dermatophytosis than the men The Public Health Service surveys however did not show any significant difference in sex incidence

During the war it was found that 8 per cent of all hospital admissions in the Army and the Navy were for cutaneous disease and that dermatophytosis ran second on the list

According to Weidman and his associates[4], the estimates of Peck *et al*[2] of the incidence of clinically active dermatophytosis of the feet is conservative They maintain that approximately 65 per cent of the population is affected Children below the age of 10 on the whole have a very low incidence of foot mycoses

swept westward and it is now nationwide It is well known that at or shortly after puberty tinea of the scalp as it is commonly seen disappears spontaneously

Thousands of cases of tinea capitis have been examined in the last few years and the causative fungus isolated Most observers have noted that *Microsporon audum* and *Microsporon lanosum* were responsible for nearly all of the cases of scalp ringworm However the majority of observers agree that all but a few of the cases in the present epidemic were found to be infected by *M audum* The scalp ringworm due to a microsporon of animal type like *M lanosum* is associated with a large proportion of sensitization to the fungus The human type of microsporon like *M audum* is much more resistant to local therapy because of the lack of accompanying sensitization

In a recent study[2] of the 6598 pupils of grade and junior high schools in Hagerstown, approximately 8 3 per cent were discovered to have ringworm of the scalp, nearly all due to *M audum* During the period of the study, August 1944 to November 1945, the number of boys infected was 12 1 per cent of the boys examined, the number of girls infected was 2 1 per cent of the number of girls examined Most of those affected were under 12 years of age

Incidence of Trichophytin Sensitivity

To obtain an idea of fungus allergy in the general population, the trichophytin test was performed on 776 persons living in different sections of the country Of these, 57 47 per cent could be classified as negative and 42 53 per cent had a reaction which varied from one to four plus It was interesting to note that, of 558 males tested, 48 per cent showed a positive reaction Of 218 females tested, 29 96 per cent showed a positive reaction This was of interest because it was found that there were practically as many women as men with clinically positive evidence of fungus infection of the feet The statement is frequently made that a positive trichophytin reaction is only an indication that the patient has once had a fungus infection and gives little information about the present activity of the infection Our analysis of the results of the trichophytin test indicates that the trichophytin test shows a greater number of positive reactions in patients with active fungus infection of the feet than those who were classified as clinically negative To some extent the degree of reaction seems to bear a relation to the clinical activity of the infection Lewis and Hopper found that 60 per cent of their patients with *T gypseum* infection of the feet reacted to trichophytin The number of trichophytin reactions rose to 87 per cent when definite clinical activity was present

In a recent study by Peck *et al*,[7] 406 adults living in and around New York were tested with trichophytin Of these, 27 4 per cent were found to be positive There were 250 males, with 36 9 per cent positive reactions, and 156 females who had 14 2 per cent positive reactions Also 101 children under 12 years of age were tested with trichophytin and there was no positive trichophytin test in any of these cases

Nature of the Trichophytin Antigens

Trichophytin may be defined as an extract of fungi which is used both for diagnosis and treatment It has been firmly established by many investigators, since the original preparation of trichophytin in 1902 by Plato and Neisser that the positive reaction follc ng the intracutaneous administration of this substance is due to a [illegible] vity resulting from a fungus—that is a tr [illegible]

Fungi of the *Epi* [illegible] *hyton*, [illegible] iera contain a general sensitizing factor, [illegible] ient [illegible] *Trichophyton*, in whom a hypersensitiv [illegible] will [illegible] tr [illegible] in reaction with [illegible] of tl [illegible] Jadassohn, S [illegible] ad [illegible]

ant which may not be present in any other members of the group Because the reaction of sensitivity may be limited to this specific excitant one may occasionally obtain a negative reaction to a trichophytin test even in the presence of ids if the specific trichophytin used lacks this substance

Trichophytin as it is commercially available is a complex material probably containing a number of different antigens the potency of which is dependent to a great extent on the method of preparation [9] Therefore it is of prime importance that before it is used some understanding be obtained of the method of preparation of the particular extract used for testing or treatment

The usual method of preparation of trichophytin consists of inoculation of Sabouraud s bouillon with *T gypseum* or *T interdigitale* contained in a large Erlenmeyer or Roux flask at a pH of about 6 5 to 7 Growth is allowed to proceed at room temperature for 10 to 12 weeks until a large surface pellicle is formed The pellicle is then tricherated with sand in a mortar after preliminary freezing with solid carbon dioxide This freezing facilitates the breaking up of the mass of mycelium and spores A sufficient amount of the bouillon is added to make a sludge The semiliquid mass is placed in a shaking machine for 24 hours It is then kept in an incubator for another 24 hours and again shaken for 24 hours The mass of growth and broth is filtered through several thicknesses of filter paper and the filter is passed through a Seitz filter The final filtrate may have to be diluted before use in order to avoid nonspecific reactions It has been used for cutaneous tests and for treatment Usually 0 5 per cent phenol is added as a preservative

There are 3 commercial fungus extracts available which are all called trichophytin They are obtained by different methods of manufacture and are therefore not similar either quantitatively or qualitatively It is obvious that since we lack a method of standardization the results obtained by the use of these different extracts are not comparable

Bloch Labouchere and Schaaf [10] in 1925 isolated from ordinary trichophytin a starch like protein free polysaccharide Nitrogen however was still present after purification These authors were able to demonstrate that this polysaccharide will still elicit positive reactions in patients sensitive to ordinary trichophytin In addition they demonstrated that it produced specific cutaneous reactions in guinea pigs infected by fungi and elicited a Shultz Dale reaction in the uterine strip of guinea pigs previously infected with fungi These experiments indicate that most likely the polysaccharide was an active and specific principle of trichophytin

Da Fonseca and his collaborators [1] prepared what they called a classo-vaccine from over 300 strains of fungi including *Trichophyton Microsporon Epidermophyton* and *Endodermophyton* This was protein free and seemed to consist of a carbohydrate which closely resembled the preparation of Bloch The Classo extract was said to be efficacious in treatment of many kinds of fungus infections but it did not contain the skin reactive substance capable of eliciting a positive reaction to a trichophytin test Sulzberger Lewis and Wise[1] tested the Classo extract in a large series of patients and

were able to show that it did contain the skin test principle. Da Fonseca and his collaborators had another preparation which was used exclusively for intradermal testing and had no therapeutic action.

Peck and Glick[9] *in a series of experiments were able to show that the* skin reactive factor which is responsible for the elicitation of a positive cutaneous reaction to trichophytin is found in both the bouillon and the pellicle of a culture of *T. gypseum* on Sabouraud's bouillon. This skin reactive factor could be demonstrated to be present in the pellicle from the latter's earliest appearance. In their experiments, enough pellicle material could be gathered in 7 days to demonstrate this specific factor. Such concentrations remained at approximately the same level throughout the whole period of the experiment, that is 66 days.

In the bouillon, however, the concentration of the skin reactive factor increased with the age of the culture under ordinary growth conditions. It could be demonstrated to be present in 7 days, but reached its maximum concentration in 40 to 50 days under varied experimental conditions.

In further experimentation Peck, Glick, and Weissbard[13] were able to show the following results. When Sabouraud's bouillon was acidified with hydrochloric acid or phosphate (McIlvane) buffers and inoculated with *T. gypseum*, there was a rise in pH. In approximately 75 to 80 days it *attained a pH of 8.6 and after that the pH remained relatively unchanged.* With the rise in the pH value, the skin reactive principle began to appear and, within limits, its concentration roughly paralleled the rise of the hydrogen ion concentration. A rise in pH from 4.0 to 6.0, even if it occurred in a relatively short time, usually indicated a concentration of skin test principle which was in excess of that found in equivalent amount of Lederle 1-30 trichophytin.

When Sabouraud's bouillon was initiated on the alkaline side by the addition of 1-5 phosphate buffers at a pH of 9.0 or 10.0 or even somewhat lower and inoculated with *T. gypseum*, the hydrogen ion concentration usually [illegible]

also was roughly parallel to the rise in the pH value.

T. purpureum formed less skin test principles than *T. gypseum* under [illegible] This is of interest, since it is known [illegible] usually not associated with a positive [illegible] en content of trichophytin was found to bear no relation to the amount of skin test factor present in bouillon. They were able to prepare trichophytin fractions in which apparently there was very little or no skin test principle. Such trichophytins were shown to be extremely effective in producing rapid desensitization in suitable patients without any local or focal reactions. The experiments cited suggest that the skin test factor is not necessarily identical with the desensitizing principle. Apparently some separation of one of these two factors has been accomplished.

Peck and Hewitt,[14] in 1945, were apparently able to demonstrate that several members of the group of fungi occurring in clinical lesions of dermatophytosis were found to elaborate a factor antagonistic to certain other microorganisms

The Significance of Dermatophytosis as an Occupational Health Problem

separately as the cause of absenteeism in the published statistics by the U S Public Health Service It was a common impression among dermatologists and industrial physicians that not only did dermatophytosis contribute considerably to lost time in industry but the presence of a fungus infection especially of the feet, predisposed workers to other allergic contact dermatoses

Gafafer[15] listed the frequency and duration of disabilities causing absence from work among the employees of a large public utility for a five year period He found that there were only 0 0142 calendar days disability per male reported for mycosis of the feet as against 8 836 calendar days disability per male from all disabilities

When the incidence of dermatophytosis of the feet was studied in industrial plants in many sections of the country it was shown that the incidence of dermatophytosis was high in the plant population examined but not so high as we were led to believe

It was important first of all, to determine whether dermatophytosis was an industrial disease In many industrial plants showers are compulsory after work If shower room flooring was an important source of fungus infections of the feet the incidence of this disease should be higher among those workers taking compulsory showers than among those in the same plant who did not The result of the investigation demonstrated that while it was possible under certain special conditions to deposit fungi from infected feet and thus give the possibility of infecting shower flooring the chance of doing so under actual conditions were small A much more likely

cially in those cases where the worker was on his feet a great deal increased sweating of the feet due to such shoes the wearing of heavy wool socks and exposure to insoluble oil which saturated the shoes and the socks and interfered with the evaporation of sweat all promoted maceration between the toes and on the soles and thus aggravated a pre-existing fungus infection

Peck *et al*[2] also carried out a study of the incidence of a fungus infection

in approximately 800 industrial workers and its relation to occupational dermatoses Of these workers, approximately 43 per cent showed a positive trichophytin test, indicating sensitivity to fungi and/or their products Among these cases, however, there were so few cases of allergic dermatoses that no conclusions could be drawn as to the relationship of trichophytin

trial population If trichophytin sensitization as such played a role in the development of allergic contact dermatitis, the number of positive trichophytin cases should have been higher in this group than in those who did not have a sensitivity to the materials which they contacted

It could be concluded from these studies that fungus infections play a minor role as causes of absenteeism, and sensitivity to fungi, while it is of frequent occurrence, plays no important role as a predisposing factor to the acquisition of other allergic contact dermatoses

The Relationship between Fungus Infections and Penicillin Sensitivity

introduction of the antibiotics into therapeutics, however, the importance of the fungus antigens as causes of allergic reaction has immeasurably increased

urt

sic

The urticaria and erythemas, together with joint pain, and fever in some instances, comprise the commonest allergic reactions to penicillin The induced urticarial form of penicillin allergy is often temporary, even transient in character This is an induced sensitivity and requires a definite incubation period varying from five days to three weeks In a recent study

sensitivity

tic

latent and active stage

presence of a positive 48 hour penicillin skin test without any history of previous penicillin administration Attention to the so called "spontaneous" positive skin test was first drawn by Welch and Rostenberg The active stage, based upon the pre-existing latent sensitivity, appears after exposure to penicillin and resembles the trichophytid because it is charac

terized by an erythemato vesicular eruption which tends to localize prima

Rostenberg[16] in a small series tested found an incidence of 5 per cent Sixty five children below 12 years of age were tested and none showed a positive reaction Similar observations were reported by Cormia and Lewis[17]

The work of Peck and Hewitt[14] explains the mechanism by which dermatophytons may induce a positive penicillin test and ultimately lead to reactions to penicillin which resemble those of the trichophytids The results of their investigations as previously cited showed that the common pathogenic fungi mainly responsible for many of the dermatomycoses are capable of producing an antibiotic possessing many of the properties of penicillin In the course of a fungus infection of the skin there are a number of antigens elaborated by the infectious agent One of those is responsible for the positive trichophytin test and trichophytin sensitivity and another leads to the so-called spontaneous penicillin test and represents the latent phase of penicillin sensitivity

The relationship of the incidence of the spontaneous positive penicillin test to the trichophytin reaction is of interest The trichophytin test carried out simultaneously in the patients who have not received penicillin treatment showed positive reactions in 33 3 per cent Among the penicillin positive patients there were 60 per cent who had showed a positive tricho

per cent among trichophytin negative persons Additional evidence of the relationship between previous fungus infection of the skin and trichophytin sensitivity on the one hand and spontaneous penicillin sensitivity on the other, is found in our observations on 165 children ranging in age from 2 months to 12 years who had never received penicillin In none of these was either the penicillin or trichophytin test positive These negative findings are in accord with the known fact that even if present fungus infections in the form of dermatophytosis rarely produce sensitivity to trichophytin in children below 12 years of age

It has been shown that in spite of practically the same incidence of fungus infection among males and females there was a much higher percentage of males who acquired a sensitivity than females This trend also applies to penicillin sensitivity Among 130 patients who received penicillin there were 32 with skin eruptions 34 2 per cent of all males developing them as against only 8 3 per cent of females

Among 276 adults whom we treated who had never received penicillin there were 168 males, of whom 6 05 per cent showed the so-called spontaneous positive reaction Of 108 females only 4 or 3 7 per cent showed a positive reaction

The active stage of penicillin sensitivity occurs during treatment Unlike

the induced form, this reaction can occur on the first day or two or even on the first administration of penicillin We had seven such cases All were males and the trichophytin and penicillin tests were positive in each case The much higher incidence of positive penicillin and trichophytin tests in this group than in the urticarial form of reaction is indicative of pre-existing sensitivity and its relation to fungus disease Patients with this type of penicillin sensitivity usually have persistent penicillin allergy of varying degrees

There is no common antigen between crystallin penicillin and trichophytin We[18] have been able to show that the antigenicity of penicillin closely parallels antibiotic potency In contrast with penicillin, trichophytin is much more stable and will act as an antigen after long standing and after heating As a final evidence that the penicillin and trichophytin [illegible]

Experimental evidences[15] suggest that streptomycin, as at present commercially available, contains an antigen closely related to penicillin Penicillin itself may be present in small amounts in streptomycin Also, there is a possibility that streptomycin and trichophytin contain a common an[illegible]

[illegible]

there is no question but that a great deal of work should be carried out in obtaining as pure a product as possible However, if the origin of the an[illegible]

[illegible]

Bibliography

1 PECK S M 1936 Allergic manifestations of fungus disease N Y State J of Med 36 1

2 PECK S M I BOTVINICK & L SCHWARTZ 1944 Dermatophytoses in industry Arch Dermat & Syph 50 170–178

3 OSBORNE E D & B S HITCHCOCK 1931 Prophylaxis of ringworm of the feet J A M A 97 453

4 WEIDMAN F D C W EMMONS J G HOPKINS, & G M LEWIS 1945 The war and dermatophytosis J A M A 128 805

5a PECK S M 1947 Suggested methods for the control of the present epidemic of tinea of the scalp Penn Med J 50 569

5b LEWIS G M M E HOPPER & F REISS 1946 Ringworm of the scalp J A M A 132 62

5c LIVINGOOD C S & D M PILSBURY 1941 Ringworm of the scalp J Invest Dermat 4 43
[illegible] & S E FRAZIER Con Pub Health Bull No 294
1948 Problems in penicillin
An [illegible] che untersuch h 171 461
1
11 DAFONSECA O JR A F DE AREA LEAO [illegible] GONSALES &
12 St [illegible]
13 P [illegible]
14 P [illegible]
15 GAFAFER W M 1943 [illegible] from work among the employees of a rubber [illegible] 1938 1942 Pub Health Bull 88 1554
16a WELCH H & A ROSTENBERG JR 1944 H[illegible] of the tuberculin type to crystalline penicillin sodium J A M A 126 [illegible]
16 ROSTENBERG A JR & H WELCH 1945 A study of the types of hypersensitivity induced by penicillin Am J Med 210 1 8
17 CORMIA F E & G M LEWIS 1946 Experimental aspects of penicillin sensitization with special reference to conjoint sen[illegible] on superficial fungous disease J Invest Dermat 7 3 5
18 PECK S M S SIEGAL & R BERGAMY 194[illegible] Immunologic relationships of the antibiotics and trichophytin Clinical observations and animal experiments J Invest Dermat 9 4
19 PECK S M & S SIEGAL 1947 Successful desensitization in penicillin sensitivity J A M A 134 1546-1547

PRACTICAL APPLICATIONS OF IMMUNOLOGIC PRINCIPLES IN THE DIAGNOSIS AND TREATMENT OF FUNGUS INFECTIONS

By Donald S Martin*

Department of Preventive Medicine and Public Health Duke University School of Medicine and Duke Hospital Durham North Carolina

The immunologic reactions in most fungus diseases differ markedly from those usually encountered in the acute bacterial, viral, or Rickettsial infections In general, the fungi causing systemic diseases are relatively non

sensitivity of the antigens which we have employed for the cutaneous and serologic tests

Our experience has been limited largely to cases of North American blastomycosis and pulmonary moniliasis The test materials used in these diseases have consisted almost entirely of crude saline suspensions of heat killed fungi Most of the patients who came to the clinic had had their disease for

ing the type of therapy The following data were obtained by [illegible] tion of the hospital records of 24 cases of systemic blastomycosis and 20 patients with the cutaneous form of the disease

With a few exceptions, the skin testing material used in blastomycosis consisted of a vaccine made from a yeast like phase of *Blastomyces dermatitidis* cultivated on blood agar at 37°C The vaccine was standardized by making a 1–1000 suspension of the packed cells in physiologic saline and then sterilized by heating to 60°C for two hours

Of the twenty patients with cutaneous blastomycosis fifteen were skin tested with vaccine and all fifteen reacted positively Of the twenty four *patients with the systemic type of infection, nineteen were skin tested*, but only nine, or about one half, of the patients reacted positively The remaining ten patients gave negative responses to intracutaneous injections of the vaccine Of numerous skin tests on patients without blastomycosis, none has given a positive test

The relative specificity of the skin reaction is in contrast to the experience of others who have tested infected animals and shown cross reactions be-

[1, 2] It

e made

ist es-

sentially of filtrates of cultures grown for a long time on a broth medium Such extracts may be more sensitive in eliciting skin reactivity, or nonspecific factors may be contained in the filtrate, accounting for the cross reactions

* Present address University of Puerto Rico School of Medicine San Juan P R

observed in animals. The great variations in potency among different lots of such extracts has been emphasized by Howell.[2] Most probably, the apparent specificity of the reaction to our vaccine is accounted for by the small amount of antigen present in the crude vaccine. Such skin testing materials probably would be far too insensitive to be of any value in a survey of population groups for evidences of a previous infection but from the standpoint of the patient with active disease, the results have proven to be of value. In extremely hypersensitive patients, sterile abscesses are found at the site of injection.

From the standpoint of therapy, a positive skin test in skin blastomycosis is indication for desensitization treatment with vaccine before applying X ray treatments or administering iodides. Response to small doses of X rays is much more rapid if the patient is partially desensitized, and we have seen skin lesions spread rapidly in a hypersensitive patient who was given large doses of potassium iodide without preliminary vaccine treatment.

The low percentage of positive skin tests to *Blastomyces* vaccine obtained in systemic blastomycosis has been interpreted as indicating a state of anergy in such patients. Of the nine patients with positive skin tests, three died, four showed marked improvement and two were classified as cured, one of these patients remaining well for the past thirteen years. Of the ten patients with negative skin tests, four have died, two showed no improvement when they left the hospital and one was not followed. Four patients improved, two of them following treatment with immune serum. In our experience, a negative skin test to the vaccine suggests a more serious prognosis than does a positive reaction.

The present complement fixation tests for *Blastomyces dermatitidis* are unsatisfactory. In most of these tests we have used as antigen a living suspension of yeast-like organisms. The dose of antigen employed in each test was based on the anticomplementary titer of the suspension, a property which has no relationship to the combining power of the antigen. The large cells of *Blastomyces* in such a suspension provide a relatively low surface to volume ratio and it is not surprising that negative or low titers are obtained in clinical infections. Serologic tests were done on only seven patients with cutaneous blastomycosis, and five of them gave positive results although the titers were relatively low. Eight of seventeen systemic cases gave positive complement fixation tests, and, in general, the titers were higher in the systemic infection than in patients with only cutaneous involvement.

It was suggested at one time[4] that the prognosis of the patient could be correlated with the reaction to the skin test and the serum antibody titer. However, as more cases have been studied so much variation has been found that we feel less inclined to attach too much prognostic significance to the results of the tests alone. It is felt that if a more sensitive and better standardized complement fixing antigen could be developed, the serum antibody titer might have significance.

Preliminary experiments have indicated that a protein fraction, precipitated from a yeastlike *Blastomyces* cell, ruptured by sonic waves, is an excellent complement fixing antigen. Interestingly enough, the polysaccharide precipitated from these ruptured cells fixes guinea pig complement with im-

mune rabbit sera but not with sera of patients with blactomycosis This finding corresponds with the well known results of complement fixation tests with pneumococcus polysaccharide as antigen, in which fixation is obtained when the rabbit antibody guinea pig complement polysaccharide antigen system is used, but not when human antibody is substituted for the rabbit antibody The extracted protein fractions of *Blastomyces* however, will fix guinea pig complement with both rabbit and human antibodies

We have made it a practice to skin test all patients with pulmonary moniliasis The skin test itself is of no value as a diagnostic agent because of the extremely high incidence of positive skin tests in the general population *Candida albicans* occurs so frequently in the oral cavities, feces *etc* of normal patients that the finding of a positive skin test in a normal individual should occasion no surprise It must be emphasized that a diagnosis of pulmonary moniliasis can be made only with difficulty, because *C albicans* is such a common secondary invader in many types of chronic pulmonary disease However once the diagnosis of pulmonary moniliasis has been established a skin test should be done in order to plan a course of therapy We have seen hypersensitive patients clear up entirely after injection of desensitizing doses of vaccine On the other hand a dramatic improvement followed antiserum therapy in a case of pulmonary moniliasis in which the skin test to vaccine was negative

Although excellent agglutination reactions are obtained easily with hyper immune rabbit sera, it has been our experience that the agglutination reactions with human sera are very unsatisfactory even in patients with definite clinical manifestations of moniliasis The problem was studied by Mrs Rees in this laboratory, and it was found that clear-cut agglutination occurred with human sera if the yeast cells were washed twice in saline before setting [illegible]

serum titer by one to two tubes but also made a great change in the qualitative appearance of the agglutinated clumps Agglutination of the washed cells resulted in the formation of definite large clumps which were distinguished easily from the controls

[illegible]

218 sera sent to the serologic laboratory for Wassermann tests it was found that 65 per cent of these sera gave positive agglutination reactions for *C albicans* in a titer of 1–20 or higher Sixty five of eighty six females (75 per cent) and seventy-eight of 132 males (57 per cent) gave positive reactions The highest age and sex incidence (87 per cent) was found in females in the 21 to 40 age group

The differences in results obtained with unwashed and washed cells were investigated by testing 134 sera using both methods Of 100 sera which gave negative reactions with unwashed suspensions, 53 were negative also

with the washed cells Of the remaining 47 sera, 15 gave a titer of 1-20, 20 of 1-40, 8 of 1-80, and 4 of 1-160 with the washed cells

Summary and Conclusions

It is felt that future efforts should be directed toward the development of more sensitive and better standardized antigens so that the results of serologic and skin tests can be evaluated more accurately and correlated with the clinical picture of the infected patient Preliminary experiments have indicated that the disintegration of pathogenic fungi by the sonic oscillator may permit the extraction of relatively pure testing substances It remains to be seen whether or not the development of more sensitive *Blastomyces dermatitidis* antigens will result in the finding of evidence of subclinical infections in endemic areas such as North Carolina

References

EMMONS, C W, B J OLSON & W W ELDRIDGE 1945 Studies of the role of fungi in pulmonary disease I Cross reactions of histoplasmin Pub Health Rep 60 385

HOWELL ARDEN JR 1947 Studies of fungus antigens I Quantitative studies of cross reactions between histoplasmin and blastomycin in guinea pigs Pub Health Rep 62 631

MARTIN, D S 1941 The practical application of some immunologic principles to the diagnosis and treatment of certain fungus infections J Invest Derm 4 471

HISTOPLASMIN SKIN TEST

By Alexander M Iams

Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota

In the past five years, histoplasmosis has assumed a more important role in American medicine than most diseases of such apparent clinical rarity Since Darling's first case of histoplasmosis was reported in 1906, over ninety proven cases of the disease have appeared in the literature [1] In spite of this small number of reported cases, a great deal of interest has been aroused by a series of papers concerning the histoplasmin skin test and its relationship to pulmonary calcification

In 1943, Long and Stearns,[2] in a review of 53,400 induction center roentgenograms of the army inductees, noted that "the incidence of calcified lesions *presumed to represent healed tuberculosis corresponds to the now well known* pattern of regional differences in the United States High incidence, (over 15 per cent) was noted in a region bounded roughly by Fort Oglethorpe, Georgia, Jefferson Barracks, Missouri, Little Rock, Arkansas, and Columbus, Ohio In general, the calcifications noted in the films of men from this region were considerably larger and more extensive than those in men from other parts of the country Also, disseminated miliary calcifications variously believed to represent healed residuals of a post primary hematogenous dissemination of tuberculous, or perhaps in some cases, a healed fungus infection of the lungs seemed relatively more frequent in this area "

Other authors[3–7] previously noted that, in the area where there was found a high incidence of pulmonary calcifications, there was also a high percentage of negative Mantoux reactors in these same patients

specificity, stated

Indiana, Tennes

w York may have

This is the area *of pulmonary calcifications in tuberculous negative persons and* is also the endemic area of histoplasmosis "

Some investigators[3–7, 9, 10] feel that the negative tuberculin reactions in patients with pulmonary calcifications are due to loss of sensitivity with the healing of the tuberculous lesion This has not been generally accepted, however In 1945, and later in 1946, Palmer,[11, 12] following the suggestions

… reactions of 10,580

ating

tors

Christie and Peterson,[13–15] in a series of three articles …), re-

… from the Mis-

rs ranging

d Palmer[16]

Of these,

Furcolow *et al*[17] reported the longest series of more than [illegible],000 tests from

Kansas City and had findings comparable to those of Christie and Peterson

With all the interest aroused by these observations and because no studies of the histoplasmin skin test in children have been undertaken in Minneapolis I thought it would be of value to make a random sampling of the children in the Twin Cities At the present our study of children in the Minneapolis area is still inadequate to show anything but trends A more complete survey will be reported later These tests compared with a series of tests on adults in Minneapolis being carried out by Johnson [15] combined with other reported surveys and analyzed as to age sex race tuberculin reaction, and pulmonary calcification might be of interest

Material and Methods of Present Study

Through the cooperation of the Department of Pediatrics University of Minnesota, the histoplasmin skin test was introduced as a routine test on the pediatric floors at the University Hospital and Minneapolis General Hospital Other studies have been done at the Catholic Boys Home and Northwestern Hospital Minneapolis Tuberculin studies were also done at the same time Roentgenograms of the chest have been planned for any positive reactors The antigen used was kindly furnished by Dr C W Emmons of the National Institute of Health It was from the lot H_3 The dilution of 1 100 was used and 0 1 cc was injected intradermally A test was considered positive if the area of induration was more than 5 mm in diameter in 48 hours

So far we have done 210 tests of which only two were positive or 1 0 per cent This is in contrast to the figures of Johnson[15] of this city who in 500 adult cases, found 41 (8 0 per cent) positive reactors His figures agree with those of Palmer [11] It is also significant that he used a dilution of 1 1000

Thus our figures are much lower so far than other investigators and if any conclusion can be drawn it might be said that the histoplasmin sensitivity in this area is developed at a later age than 15 years

Review of Other Studies

The Antigen Van Pernis Bensen and Holinger [8] first prepared histoplasmin from dextrose broth cultures of the fungus Zarafonetis *et al* [20] also reported the preparation and use of histoplasmin antigen However most of the recent studies have been done with antigens prepared by either C W Emmons or Amos Christie The former prepares[21] his antigen from cultures of *H capsulatum* grown on a medium similar to that used for tuberculin containing asparagine dipotassium phosphate sodium citrate magnesium sulfate ferric citrate dextrose glycerine and water A three-month culture of the fungus is filtered through a Berkfield N filter and tested for sterility Merthiolate is added in concentration of 1 10 000 Christie s method of preparation has not been published but I assume it is similar to that of Emmons

The Test The material is injected in dilutions of 1 100 to 1 1000 depending on the investigator 0 1 cc of the substance is injected intradermally

HISTOPLASMIN SKIN TEST

By Alexander M Iams

Department of Pediatrics University of Minnesota, Minneapolis, Minnesota

In the past five years, histoplasmosis has assumed a more important role in American medicine than most diseases of such apparent clinical rarity Since Darling's first case of histoplasmosis was reported in 1906, over ninety proven cases of the disease have appeared in the literature [1] In spite of this small number of reported cases, a great deal of interest has been aroused by a series of papers concerning the histoplasmin skin test and its relationship to pulmonary calcification

In 1943, Long and Stearns,[2] in a review of 53,400 induction-center roentgenograms of the army inductees noted that "the incidence of calcified lesions presumed to represent healed tuberculosis corresponds to the now well known pattern of regional differences in the United States High incidence, (over 15 per cent) was noted in a region bounded roughly by Fort Oglethorpe Georgia Jefferson Barracks, Missouri, Little Rock, Arkansas, and Columbus, Ohio In general the calcifications noted in the films of men from this region were considerably larger and more extensive than those in men from other parts of the country Also, disseminated miliary calcifications variously believed to represent healed residuals of a post primary hematogenous dissemination of tuberculous, or perhaps in some cases, a healed fungus infection of the lungs seemed relatively more frequent in this area "

Other authors[3–7] previously noted that, in the area where there was found a high incidence of pulmonary calcifications, there was also a high percentage [illegible] pecificity, stated [illegible] ndiana, Tennessee, Kentucky, Ohio West Virginia, Pennsylvania, and New York may have borderline positives while a few are undoubtedly positive This is the area of pulmonary calcifications in tuberculous negative persons and is also the endemic area of histoplasmosis "

Some investigators[4–7, 9, 10] feel that the negative tuberculin reactions in patients with pulmonary calcifications are due to loss of sensitivity with the healing of the tuberculous lesion This has not been generally accepted however In 1945 and later in 1946, Palmer,[11, 12] following the suggestions of Smith and of Christie reported the histoplasmin skin reactions of 10 580 nurses from all sections of the United States In an area closely simulating that described by Long and Stearns,[2] 68 3 per cent were positive reactors Christie and Peterson [13–15] in a series of three articles in 1945 and 1946, reported 2,032 histoplasmin skin reactions, most of which were from the Mississippi River basin [illegible] reactors ranging from 59 4 per cent to [illegible] reported a series of [illegible] 494 were histoplasmin positive, tuberculin negative

Furcolow *et al* [17] reported the longest series of more than 17 000 tests from

Kansas City and had findings comparable to those of Christie and Peterson With all the interest aroused by these observations and because no studies of the histoplasmin skin test in children have been undertaken in Minneapolis, I thought it would be of value to make a random sampling of the children in the Twin Cities At the present, our study of children in the Minneapolis area is still inadequate to show anything but trends A more complete survey will be reported later These tests compared with a series

Material and Methods of Present Study

Through the cooperation of the Department of Pediatrics University of Minnesota, the histoplasmin skin test was introduced as a routine test on the pediatric floors at the University Hospital and Minneapolis General Hospital Other studies have been done at the Catholic Boys' Home and Northwestern Hospital, Minneapolis Tuberculin studies were also done at the same time Roentgenograms of the chest have been planned for any positive reactors The antigen used was kindly furnished by Dr C W Emmons of the National Institute of Health It was from the lot H_3 The dilution of 1.100 was used and 0 1 cc was injected intradermally A test was considered positive if the area of induration was more than 5 mm in diameter in 48 hours

So far, we have done 210 tests, of which only two were positive, or 1 0 per cent This is in contrast to the figures of Johnson[18] of this city who, in 500 adult cases found 41 (8 0 per cent) positive reactors His figures agree with those of Palmer[11] It is also significant that he used a dilution of 1:1000

Thus, our figures are much lower so far than other investigators, and if any conclusion can be drawn, it might be said that the histoplasmin sensitivity in this area is developed at a later age than 15 years

Review of Other Studies

The Antigen Van Pernis, Bensen, and Holinger[19] first prepared histoplasmin from dextrose broth cultures of the fungus Zarafonetis *et al*[20] also reported the preparation and use of histoplasmin antigen However,

method of preparation has not been published, but I assume it is similar to that of Emmons

The Test The material is injected in dilutions of 1:100 to 1·1000, depending on the investigator, 0 1 cc of the substance is injected intradermally

HISTOPLASMIN SKIN TEST

By Alexander M Iams

Department of Pediatrics University of Minnesota Minneapolis, Minnesota

In the past five years, histoplasmosis has assumed a more important role in American medicine than most diseases of such apparent clinical rarity Since Darling s first case of histoplasmosis was reported in 1906, over ninety

to pulmonary calcification

In 1943, Long and Stearns,[2] in a review of 53,400 induction center roentgenograms of the army inductees, noted that "the incidence of calcified lesions presumed to represent healed tuberculosis corresponds to the now well known pattern of regional differences in the United States High incidence (over 15 per cent) was noted in a region bounded roughly by Fort Oglethorpe Georgia, Jefferson Barracks, Missouri, Little Rock, Arkansas, and Columbus, Ohio In general the calcifications noted in the films of men from this region were considerably larger and more extensive than those in men from other parts of the country Also, disseminated miliary calcifications variously believed to represent healed residuals of a post primary hematogenous dissemination of tuberculous, or perhaps in some cases, a healed fungus infection of the lungs, seemed relatively more frequent in this area "

Other authors[3-7] previously noted that, in the area where there was found

of pulmonary calcifications in tuberculous negative persons and is also the endemic area of histoplasmosis "

Some investigators[3-7, 9, 10] feel that the negative tuberculin reactions in patients with pulmonary calcifications are due to loss of sensitivity with the healing of the tuberculous lesion This has not been generally accepted

ssissippi River basin They found percentages of positive reactors ranging from 59 4 per cent to 83 9 per cent In 1946 and 1947, Zwerling and Palmer[16] reported a series of 698 patients with pulmonary calcification Of these 494 were histoplasmin positive, tuberculin negative

Furcolow *et al* [17] reported the longest series of more than 17,000 tests from

In a different set of test animals, however, he had entirely different percentages of positive reactors From this, he concluded that the reactions also depend on the stage of the disease at which the tests were done This he called the level of sensitivity of the animals or patients, and noted its importance in determining the titer of the antigen

He then tested the cross reactions of the animals between blastomycin, histoplasmin coccidioidin, and tuberculin As did Emmons, he found that there was a large number of cross reactions between histoplasmin and blastomycin However, in using different lots and different concentrations he found that the percentage of cross reactions depended on the lot and concentration of each antigen He felt that the number was more apparent than real and that, if the critical titer of each was determined and used they would be decreased considerably He felt that the acquirement of blastomycosis did sensitize the animals to the histoplasmin antigen since a much higher percentage reacted positively to the antigen after acquiring the disease than before He does not feel that the cross reactions are almost com plete,' as Emmons says they are

Christie[18] has deduced from his studies of the histoplasmin skin test in man that the cross reactions noted by Emmons are not seen in human sub jects

Thus, one sees that the whole field of specificity is wide open still While the test is not as specific as is coccidioidin, it is probably more specific than Emmons has shown

Results

The histoplasmin skin test has been used clinically for diagnostic purposes too few times as yet for any definite information to be gained It is the general opinion of most investigators, however, that patients with a fully developed clinical picture of the disease are anergic and give a negative re action This was my experience with a patient seen at the Mayo Clinic[1] It was interesting to note that the mother of this patient reacted very posi tively to the antigen This has also been the experience of others The only deduction to be drawn from this is that both patients were exposed to the same source of infection, but of possibly variable dosages The mother in this case showed no signs of histoplasma infection

As stated previously, the bulk of the tests reported in the literature have been done in an effort to solve the problem of the patient with pulmonary calcification and a negative tuberculin test There have been 27 780 tests *in all reported in studies of this kind As shown in* TABLE 1 *38 6 per cent* (10 732) of these were histoplasmin positive reactors and 61 4 per cent (17 018) [illegible] None of these patients showed

tuberculin tests were reported In the former group, 44 13 per cent were

and read in 48 and 72 hours Any test showing an area of induration over 5 mm is considered positive

Specificity As with all skin tests used for diagnostic purposes in medicine there have been questions raised as to the specificity of this test Van Pernis *et al*[19] first used the test both on experimental animals and on an established case of histoplasmosis in a human patient Both gave positive reactions The most important usage of the test, however, has been in the problem of pulmonary calcification, and in this the problem of specificity is of particular importance

Emmons[1] has reported the first investigation of this matter He first injected 0 1 cc of histoplasmin intradermally in dilutions of 1 10 1 100 and 1 1000 into twelve normal guinea pigs None of these reacted In four of these animals he repeated the tests and in none was there any sign of irritation or sensitization by the antigen He then tested 39 guinea pigs with experimental histoplasmosis Thirty two of these gave reactions to a dilution of 1 100 Also 9 rabbits similarly infected, gave positive reactions

to the histoplasmin antigen Of particular significance were the reactions to blastomycosis All 8 test animals were positive reactors In studying this reaction further he did titration studies of the antigens of the two fungi testing their cross reactions in experimental animals in many varying dilutions From these studies, he concluded that There seems to be an almost complete cross reaction between histoplasmin and blastomycin in experimental blastomycosis and histoplasmosis in guinea pigs "

In studying 136 hospitalized patients half of whom had atypical pulmonary lesions he found 40 4 per cent reacted positively to histoplasmin and 25 7 per cent reacted positively to blastomycin The histoplasmin test

some use in the epidemiological field, its use as a diagnostic procedure was certainly limited

He also has found cross reactions experimentally with *Candida albicans* and feels this might well explain many of the positive reactors in the reported surveys[26]

However, Howell[2] has reported the most complete study into the whole subject *Six strains of Histoplasma capsulatum* and five of *Blastomyces* were

und that

ntages of

umber of

positive reactors depends on the lot of histoplasmin used a ... lution of the antigen He recommended therefore, that the antigens be standardized preferably by employing a concentration of each lot which would detect a similar percentage of sensitized animals This concentration he called the titer which he defined as the minimum amount which would detect sensi

Age The majority of these tests reported in the literature have been applied to children Of the reported 27 780 tests 62 7 per cent have been in the age limits of birth to 18 years Furcolow [7] in reporting 16 000 school children in Kansas City found 5 per cent positive reactors at 2 years 20 per cent at 6 years 30 per cent at 8 years 50 per cent at 12 years and 65 per cent at 18 years Christie [13] reported somewhat higher percentages of positive reactors from Tennessee He found 18 per cent at 2 years 36 per cent at 5 years, 58 per cent at 10 years 80 per cent at 15 years Olson [6] reported even higher figures from Virginia but from a much smaller group He found 47 per cent at 5 years, and 82 per cent at 14 years These are all in contrast to our own figures of less than 1 per cent in all ages

As far as development of pulmonary calcification is concerned all these investigators found the percentages lagging appreciably behind the positive reactors at the same age This has been explained by the reasoning that it takes quite a bit longer to develop the calcifications than it does the sensitivity and probably it takes several repeated invasions by the organism to cause enough calcification to be seen by X ray

TABLE 3

RELATIONSHIP OF HISTOPLASMIN AND TUBERCULIN SKIN TESTS TO PULMONARY CALCIFICATION

	H+ T+	H+ T−	H− T+	H− T−	Total
No	335	1791	137	314	257
%	13 0	69 5	5 3	12 1	16 1
Total number of tests					16 000

In the older age groups the percentages of positive reactors have ranged from 20 9 per cent reported by Palmer [11] to 85 to 90 per cent reported by Christie [13] and Furcolow [7]

Sex and Race Sex does not appear to be a factor in histoplasmin sensitivity However, Furcolow [7] is the only one who has reported this aspect in any detail More investigation is needed before a definite statement can be made As far as race is concerned the white race seems to be more sensitive than the colored race but this is not enough to be of any significance

Location Of the 27 780 tests reported approximately 20 459 (73 7 per cent) have been from the mid Central states and most of these from Kansas City or Tennessee Thus the percentages may not be an exact index of the whole picture In the few studies that have included a wider selection however the percentages have been about the same Palmer [11] as stated before in this paper found 68 3 per cent of positive reactors in these mid Central states The Southwest and California fell far under this percentage The Southeastern and Northwestern areas showed the lowest percentage Christie and Peterson [15] noted a similar distribution but their percentages were appreciably higher in the outlying states No studies were made in the Northwest, West or Southwest, however

One can see that the areas of highest histoplasmin reaction closely corre

histoplasmin positive and 55 87 per cent were negative, thus closely following those in the larger group Histoplasmin positive, tuberculin positive reactors were only 4 05 per cent, while histoplasmin positive, tuberculin negative were 40 56 per cent Of particular interest is the fact that only 4 36 per cent were histoplasmin negative, tuberculin positive, while 51 51 per cent were negative to both tests Thus, of the whole group, while 44 13 per cent were positive to histoplasmin, only 8 41 per cent were tuberculin reactors As will be shown later, this percentage is not a true index of the population as a whole, since most of these tests were reported from one section, namely the central Mississippi River basin In these present statistics those tests that were necessarily not included were those of Palmer,[11] which

TABLE 1
TOTAL HISTOPLASMIN TESTS REPORTED IN LITERATURE

H+ T+	H+ T−	H+ *T?	H− T+	H− T−	H− T?
% 2 8	27 0	8 9	3 0	34 8	23 7
Total No %		10 732 38 6	Total No %		17 048 61 4
		Total—27 780			

* Tuberculin results not reported

TABLE 2
RELATIONSHIP OF HISTOPLASMIN AND TUBERCULIN TESTS

%	H+	H−	Total
T+	4 05	4 36	8 41
T−	40 56	51 51	91 53
Total	44 13	55 87	100
Total number of cases			18 746

[illegible] percentage of positive re- Thus, these present sta[illegible]es only in the comparison [illegible]y, Palmer did not report

TABLE 3 shows the histoplasmin tuberculin reactions of 2,577 (16 1 per cent) patients reported from a group of 16 006 patients studied [13, 11, 18, 23, 24] All these patients showed varying degrees of pulmonary calcification by roentgenogram Here, it is interesting to note that 82 5 per cent reacted positively to the histoplasmin test and 18 3 per cent reacted positively to the tuberculin test Thus, the positive reactors are four times as great as the percentages of Palmer[11] (20 9 per cent) and twice as great as the over all [illegible] reported in this paper The percentage of tuberculin reactors

skin testing as a means of establishing an epidemiological index of tuberculous infection Am J P H 29 25

5 Olsen B J W H Wright & M O Nolan 1941 Epidemiological study of calcified pulmonary lesions in an Ohio county Pub Heal Rep 56 2105

6 Lumsden L L & W P Dearing 1940 Epidemiological studies of tuberculosis Am J P H 30 219

7 Crimm P D & D M Short 1939 Tuberculin anergy in cases with pulmonary calcification Am Rev Tbc 39 64

8 Smith C E 1943 Coccidioidomycosis Med Cl N Am 27 790

9 Norval, M A 1945 Tuberculin reaction in children with a Ghon complex review of one hundred twelve cases Am J Dis Child 70 13

10 Feldman W H & H F Helmholz 1945 Infectivity of the primary complex of tuberculosis of childhood Am J Dis Child 70 201 206

11 Palmer C E 1945 Nontuberculous pulmonary calcification and sensitivity to histoplasmin Pub Heal Rep 60 513 520

12 Palmer C E 1946 Geographic differences in sensitivity to histoplasmin among student nurses Pub Heal Rep 61 475–487

13 Christie A & J C Peterson 1945 Pulmonary calcification in negative reactors to tuberculin Am J P H 35 1131 1147

14 Christie A & J C Peterson 1946 Pulmonary calcification sensitivity to histoplasmin tuberculin and haplosporangin J A M A 131 658–660

15 Christie A & J C Peterson 1946 Histoplasmin sensitivity J Ped 29 417 432

16 Zwerling H B & C E Palmer 1947 Pulmonary calcification in relation to sensitivity to histoplasmin J A M A 134 691–692

17 Furcolow M L R H High & M F Allen 1946 Some epidemiological aspects of sensitivity to histoplasmin and tuberculin Pub Heal Rep 61 1132 1143

18 Johnson J W Personal communication Unpublished data

19 Van Pernis P M E Benson & P H Holinger 1941 Specific cutaneous reactions with histoplasmosis J A M A 117 436

20 Zarafonetis C J D & R B Lindberg 1941 Histoplasmosis of Darling Observations on the antigenic properties of the causative agent A preliminary report Univ Hosp Bull Ann Arbor 7 47

21 Emmons, C W B J Olson & W W Eldridge 1945 Studies of the role of fungi in pulmonary disease I Cross reactions of histoplasmin Pub Heal Rep 60 1383 1394

22 Howell A 1947 Studies of fungus antigens 1 Quantitative studies of cross reactions between histoplasmin and blastomycin in guinea pigs Pub Heal Rep 62 631–651

23 Zwerling H B & C L Palmer 1946 Pulmonary calcifications and roentgenographic observations in relation to histoplasmin and tuberculin reactions Radiology 47 59–63

24 High R H H B Zwerling & M F Furcolow 1947 Disseminated pulmonary calcification Pub Heal Rep 62 20

25 Olson B J J A Bell & C W Emmons 1947 Studies on histoplasmosis in a rural community Am J P H 37 441

26 McCleod J H C W Emmons S Ross & F G Burke 1946 Histoplasmosis A report of four cases two in Siblings Histoplasmin test and other diagnostic procedures J Ped 28 275–293

spond to the areas of tuberculin negative pulmonary calcifications as described by Long and Stearns.[2] It is from these areas also that the reports of histoplasmin positive, tuberculin negative pulmonary calcifications have come. Most of the reported cases of histoplasmosis have also been from these areas.

Discussion

The present status of the histoplasmin skin test has been presented. So far a great deal of work has been done and many things learned. A relationship has been found to exist between the sensitivity to histoplasmin and many patients with and without pulmonary calcification.

This sensitivity has been found to develop very early in life in a large percentage of the patients. Much material has been collected to support the theory that the disease is endemic, at least in the Mississippi River basin, and that the great majority of the patients get well without any evidence of the disease or, at most, a varying degree of pulmonary calcification. The test has been found to be of little practical diagnostic value as yet.

Much remains to be learned, however. The work of Howell[22] has shown that the test depends on the lot and dosage of the antigen used and the stage in the disease when the patient is tested. He and Emmons[21] both showed that the result of the test also depends on the general mycotic sensitivity of the patient. That is, a positive reaction does not necessarily mean a sensitivity to histoplasmin. It means that the patient is sensitive to one of several mycotic antigens, particularly blastomycin.

Howell's[22] suggestions for standardization of the antigen and determining the titer of each lot is certainly a step forward. Further clinical titrations with blastomycin are in order. The specificity of the test must be improved before any definite deductions can be made. Further studies on a more generalized nationwide basis must be done.

Emmons[21] reports the isolation of *H. capsulatum* from a hilar lymph node in a child with pulmonary calcification. None were found in 35 other cases examined. More proven cases of this kind are necessary before one can definitely prove that the calcification is caused by histoplasmosis.

Whatever is lacking in the present picture of the specificity of the histoplasmin test, the evidence of its relationship to the tuberculin negative patient with pulmonary calcification certainly cannot be ignored. This must be more than circumstantial and has opened up again the interest concerning this subject.*

Bibliography

1. IAMS, A. M. & H. M. KEITH. 1947. Histoplasmosis in infancy. J. Ped. 30: 123-128.
2. LONG, E. R. & W. H. STEARNS. 1943. Physical examination at induction. Radiology 41: 144-150.
3. GASS, R. S., R. L. GOULD, I. F. HARRISON, H. C. STEWART & W. C. WILLIAMS. 1938. Tuberculosis study in Tennessee. Am. Rev. Tbc. 38: 441.
4. LUMSDEN, L. L., W. P. DEARING & R. A. BROWN. 1939. Questionable value of

* It has been suggested to me by one of my colleagues in Minneapolis that the problem of Boeck's sarcoidosis be investigated from the point of view of histoplasmosis. I have not been able to find such a study reported as yet.

variety of structures which can be affected by the nutrients), an effect of individual amino acids on morphology can be observed readily. Consequently, the authors undertook to study some of the effects of individual amino acids on the rate, amount, and character of growth of 13 fungi pathogenic for man.

Method

The basal media employed contained 4 per cent glucose, 2 per cent (unpurified) Bacto agar, and 0.33 mg per 100 cc of each of the following vitamins: thiamin hydrochloride, riboflavin, nicotinamide, calcium *d*-pantothenate, ascorbic acid, and pyridoxine. Sodium phosphate buffer pH 7.2, was added to give a concentration of 0.016 M.

Fifteen cc aliquots of basal media were placed in Pyrex test tubes 20 × 250 mm containing sufficient amino acid (peptide or peptone) as listed below to give a concentration of 0.25 per cent of the *l* form.

ALIPHATIC ACIDS

Sulfur containing
- *l* cysteine hydrochloride
- *dl* methionine
- glutathione

Dicarboxylic acids and amides
- *l* glutamic acid
- *l* glutamine
- *l* aspartic acid
- *l* asparagine

Monobasic monocarboxylic acids
- glycine
- *l* alanine
- *l* leucine
- *dl* isoleucine
- *dl* valine
- *dl* serine
- *dl* threonine

AROMATIC, OTHER CYCLIC AND OTHER NONCYCLIC STRUCTURE

Basic amino acids
- *l* arginine
- *l* lysine
- *l* histidine

Non basic cyclic acids
- *l* tyrosine
- *l* phenylalanine
- *l* tryptophane
- *l* proline
- *l* hydroxyproline
- para aminobenzoic acid
- casein hydrolysate
- none
- peptone (Pfanstiehl)
- heart extract

The amino acid solutions used to make the media were adjusted to pH 7.0 with 1 N sodium hydroxide solution. One tube containing no added amino acid and another with peptone served as controls for comparison.

In addition, the effects of adding an extract of unheated dog or beef heart[9, 10] muscle and of combining 2, or in some cases 3, of certain amino acids were studied.

The phosphate buffer (pH 7.2) was used so as to minimize the effect of the different amino acids on the pH of the final media. Autoclaving at 15 lbs pressure for 30 minutes lowered the pH to 6.5.

Glutamine solution and the heart extract were sterilized by filtration (Berkefeld)[10] and (when used) were added to the basal media *after* the latter had been autoclaved and cooled to 40° C and not more than several hours prior to inoculation. Glutamine solution, whether by itself or as present in heart extract, is unstable to heat, especially in the presence of phosphate.[11]

Analysis of these amino acids for carbon, total nitrogen and α NH_2 nitrogen indicated that all except glutamine were of a high degree of purity. The glutamine assayed enzymatically[12] 96 per cent. This was by far the best grade available on the market.

SOME BIOCHEMICAL IMPLICATIONS FROM A STUDY OF GROWTH OF PATHOGENIC FUNGI ON MEDIA CONTAINING SINGLE AMINO ACIDS

By Reginald M Archibald and Frederick Reiss

Hospital of the Rockefeller Institute for Medical Research, New York, N Y, and Department of Biochemistry, School of Hygiene Johns Hopkins University Baltimore, Maryland Department of Dermatology and Syphilology, New York University Bellevue Medical Center, New York N Y

The combined efforts of biochemists and mycologists seldom have been brought to bear on a study of the amount and character of growth resulting from utilization of single amino acids by fungi pathogenic to humans Relatively few studies have been reported which indicate the amino acid requirements of fungi pathogenic for man Much more has been done on the nutrient requirements of nonpathogenic fungi Anderson and Emmart[1] studied the relation of certain amino acids to carbon dioxide and mycelium production of *Fusarium oxysporum* and reviewed the significant literature on amino acid metabolism by fungi up to 1934 They observed that, of the amino acids tried, aspartic acid was outstanding in its ability to produce mycelium when added to a basal medium containing glucose and inorganic salts Mosher *et al*[2] studied the effect of various minerals, carbohydrates, and amino acids in his work on the nutritional requirements of *Trichophyton interdigitale* In studying the effect of different amino acids, they omitted one or several amino acids from media which were otherwise fairly complete, and which contained minerals, vitamins, carbohydrate, and all of the other amino acids which were readily available to the workers Benham[3] reported that addition of asparagine to a medium containing oleic acid, glucose, and inorganic salts increased the yield of *Pityrosporum ovale* Robbins, Mackinnon, and Ma,[4] in studying the effect of vitamin deficiency on the growth of *Trichophyton discoides*, observed that they failed to improve growth by the addition of many amino acids to a basal medium containing peptone Robbins and Ma[5] studied in detail the growth requirements of *Trichophyton mentagrophytes (T gypseum)* They added amino acids singly and in combination to a basal medium containing inorganic anions and cations and purified agar Gottlieb[6] investigated the utilization of amino acids as a source of carbons for the growth of two nonpathogenic fungi A somewhat similar study had been conducted by Baker and Smith[7] with *Coccidioides immitis* Robbins and McVeigh[8] observed that hydroxyproline inhibited the growth of 5 pathogenic fungi, including *T mentagrophytes (T gypseum)* and *T purpureum*, when asparagine was the only other source of nitrogen in the medium The inhibition produced by hydroxyproline was largely overcome by the addition of proline but not by the addition of any one of 13 other amino acids

It has become apparent that, for a study of the effect of different amino acids on growth, the use of fungi (more highly differentiated than bacteria) has several advantages which do not attend the use of bacteria Because there is more opportunity for morphological variation (there being a greater

conclusions. However, the implication is that the pathogenic fungi studied can use the amino nitrogen of any one of several amino acids to synthesize other amino acids necessary as building blocks in the formation of the protoplasmic proteins characteristic of the species with which the media are inoculated. Any one of these organisms is capable of synthesizing proteins neces-



FIGURE 1. Colonies of *Microsporon audouini* grown for 2 weeks on media containing the indicated amino acids or peptides as a source of nitrogen. Reflected light.

sary for its growth and cell structure from any one of several amino acids. For example, the colony which grew on the media containing no amino acid other than arginine contains, presumably, a protein made up of peptide linkages between several kinds of amino acids in addition to arginine. That is, given one amino acid, these organisms can synthesize other amino acids from it. This ability to synthesize other amino acids appears to be greatest

The preparation of casein hydrolysate employed was one which has been reported to have a high content of those peptides which are included by the name Strepogenin

Each tube was inoculated with an amount of the respective fungus not exceeding the size of a small pinhead and weighing less than 2 mg [11] The species studied are *Microsporon audouini*, *Microsporon lanosum*, *Microsporon fulvum* *Trichophyton purpureum*, *Trichophyton gypseum*, *Epidermophyton inguinale*, *Achorion schoenleini*, *Monilia albicans*, *Blastomyces dermatitides*, *Histoplasma capsulatum*, *Hormodendron pedrosi*, *Sporotrichon schenki*, and *Torula histolytica*

Tubes were kept at room temperature (23–28°C) The rate of growth was determined after 7 and 14 days by measuring the height and diameter of the growth above the surface and depth and diameter of the subsurface growth Note was made of the character of the growth variants such as texture, groove and pigment formation, striking appearance of subsurface growth, appearance of colonies under a Wood's light, and microscopic changes of growth characteristics and variations

Results

Photographs of some of the 14 day cultures appear in FIGURES 1 to 11 Detailed measurements of the size of colonies and descriptions of microscopic appearances will be reported elsewhere

One of the authors has observed[11] that the viewing of colonies under ultra violet (or violet) light is a distinct aid in differentiating some species of bacteria or of nonpathogenic fungi which show identical cultural characteristics in ordinary light That is, some species of bacteria and fungi, especially when grown at room or ice box temperature, develop substances which fluoresce strongly under a Wood's light However, the pathogenic fungi here studied failed to show any striking appearance under the Wood's light

The nature and amount of growth of *T gypseum* on the various media closely resembles that reported by Robbins and Ma[8] for *T mentagrophytes*

When media containing agar, glucose, vitamins, phosphate buffers, and any *one* of several amino acids are inoculated with pathogenic fungi some growth occurs When the only amino acid present is hydroxyproline the amount of growth is very small, and may appear microscopically to consist of a few filaments extending below the surface of the medium If, however, the single amino acid present is glutamine, glutamic ac[illegible] growth is abundant When any one [illegible] amount of gro[illegible] that o[illegible] e tained [illegible] cies of [illegible] of inoculation [illegible] acid is present but also on the spe

Although fungi grown on media containing only one amino acid have not been hydrolyzed and analyzed for amino acids, they may be expected to contain proteins as postulated by Robbins and Ma [8] These proteins contain at least several and probably many amino acids Because of lack of actual analysis of hydrolysates of the growths, caution must be exercised in drawing

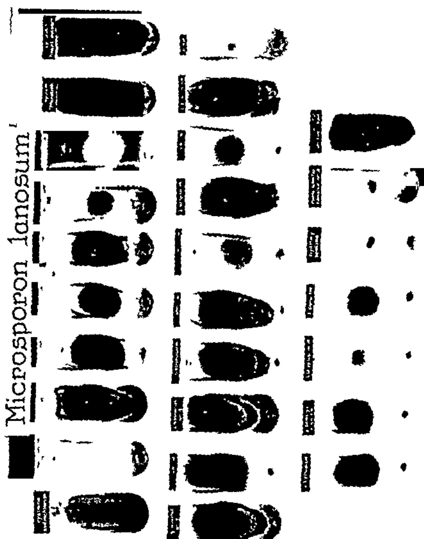


FIGURE 2. Colonies of *Microsporon lanosum* grown for 2 weeks on media containing the indicated amino acid(s) or peptide(s). Reflected light.

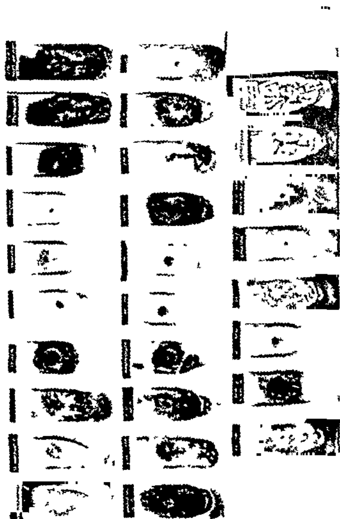


FIGURE 3 Colonies of Microsporon fulvum grown for 2 weeks on media [illegible] Reflected light

Trichophyton purpureum

FIGURE 4 Colonies of *Trichophyton purpureum* grown for 2 weeks on media containing the indicated amino acid(s) or peptide(s) Reflected light

when the nutrient amino acid is either glutamine, arginine, or glutamic acid. However, leucine, asparagine, proline, and phenylalanine likewise appear capable of acting as precursors for other amino acids. This is true to a much smaller extent of hydroxyproline, para-aminobenzoic acid, tryptophane, lysine, and methionine. Even the simplest amino acid, glycine, gives good growth with *Microsporon lanosum* and moderately good growth with *Microsporon fulvum*, although very poor growth with *Microsporon audouini*.

The only amino acids which, when present singly, support fairly good growth of *Achorion schoenleini* are arginine and leucine.

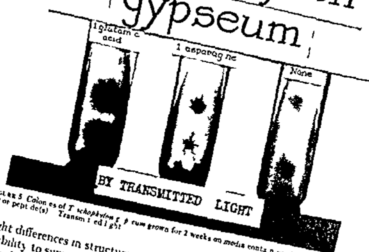


FIGURE 5. Colonies of *Trichophyton gypseum* grown for 2 weeks on media containing the indicated amino acid(s) or peptide(s). Transmitted light.

Slight differences in structure of the amino acid cause great differences in their ability to support growth of fungi. For example, growth of all species tested is better on proline than on hydroxyproline—better on leucine than on isoleucine.

Certain amino acids seem to favor the development of certain types of growth. The cultural characteristics of the colonies are in many cases determined not only by the species used but also by the amino acid present. Thus a very pronounced coarse arboration of subsurface growth is noticed with *Trichophyton gypseum* (see FIGURE 5); most marked when the amino acid supplied is asparagine.

Discussion

It will be observed that the list of several amino acids which can produce good growth has no apparent relationship to the list of amino acids which are

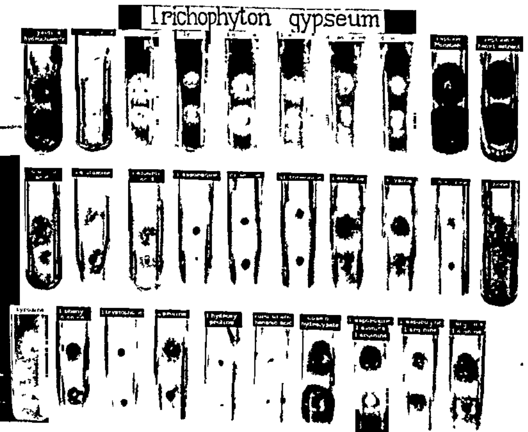


FIGURE 6 Colonies of *Trichophyton gypseum* grown for 2 weeks on media containing the indicated amino acid(s) or peptide(s) Reflected light

Blastomyces dermatitides

FIGURE 8. Colonies of *Blastomyces dermatitides* grown for 2 weeks on media containing the indicated amino acid(s) or peptide(s). Reflected light.

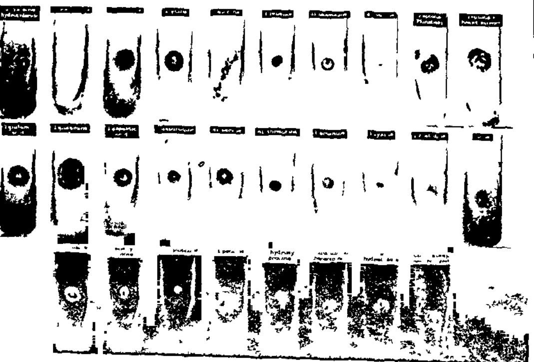

Histoplasma capsulatum



FIGURE 10. Colonies of *Sporotrichon schenki* grown for 2 weeks on media containing the indicated amino acid(s) or peptide(s). Reflected light.

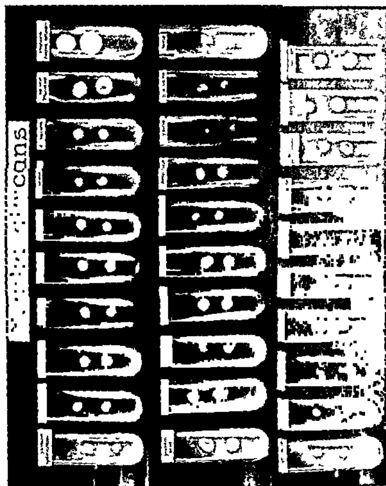


FIGURE 11 Cans [illegible] Reflected light

essential for mammalian growth Rather, it appears more closely related to the facility with which its members can participate in reactions involving transamination The ability of pathogenic fungi to synthesize many amino acids from a single amino acid should not be surprising in view of the much earlier findings of plant physiologists who studied conversion of seed proteins to glutamine and asparagine and the reconversion of this glutamine and asparagine to proteins built into the growing seedlings

There is considerable data in the literature which indicates that non pathogenic fungi and yeasts are likewise capable of synthesizing numerous amino acids from a single amino acid Kraut and Schlottmann[15] analyzed yeast protein indicating its percentage composition of histidine, lysine, cystein tryptophane and tyrosine Block and Bolling[16] indicate the amount of ten essential amino acids present in yeast powder Klose and Fevold[17] (cf also[18]) grew *Torulopsis utilis* and *Saccharomyces cerevisiae* on molasses (which is chiefly a carbohydrate medium) and found that the protein in the yeast so grown although somewhat deficient in methionine, apparently was otherwise a fairly complete protein Tumara[19] showed that a *Mycobacterium lacticola*, growing on a medium containing as the only nitrogen sources asparagine and ammonium lactate, synthesized arginine histidine lysine phenylalanine proline, valine, and tryptophane as well as other amino acids Abderhalden and Rona[20] observed that *Aspergillus niger* synthesized glycine alanine leucine, glutamic acid, and aspartic acid as they grew when the media contained any one of either potassium nitrate, glycine or glutamic acid as the nitrogen source Vorbrodt[21] isolated tyrosine leucine, and ala nine from the protein synthesized by *Aspergillus niger* growing on a medium containing only inorganic nitrogen Skinner,[22] as a result of feeding tests on rats and crude colorimetric tests concluded that the proteins synthesized by *Aspergillus niger Trichoderma konigi*, *Zygorrhynchus moelleri*, *Aspergillus oryzae Aspergillus terreus*, and *Penicillium flavo glaucum* contained all of the so-called essential amino acids even when grown on medium containing nitrogen only in the inorganic form A somewhat similar observation has been made by Takata[23] with *Aspergillus oryzae*

In general it may be said that *Hormodendron pedrosi* and the yeast like organisms studied *Monilia* and *Torula*, do not have as exacting amino acid requirements as the other species They grow fairly well when any one amino acid is present

Williams[24, 25] has conducted numerous experiments with a wide variety of fungi and observed the amount and nature of the subsurface growth obtained when his medium contained hydrolyzed hair or skin, or cysteine or when high oxygen tension was maintained Williams observed that abundant subsurface growth occurred not only when cysteine was the amino acid present but also when it was substituted for by other amino acids

Our work appears to indicate that subsurface growth is abundant when the medium is inadequate for optimum growth The greater the number of amino acids present especially if these amino acids are capable of supporting good growth the smaller is the amount of subsurface growth obtained The subsurface growth appears then, to represent an attempt on the part of the

organism to reach out for nutrient of better quality or greater variety than is present near the surface

fluenced the amount of growth However, because of the very small amount of growth found in the control tube to which no amino acid was added it can be concluded that the effect of amino acid from the inoculum and agar was almost negligible

Most of those amino acids which fail to support growth should not be regarded as inhibitors Rather, it may be assumed that the organisms are not able to convert (at sufficient rate) such amino acids to any other amino acid *which in turn could act as a precursor of all of the acids necessary for building* the proteins required for growth For example, as seen in FIGURE 1 *M*

the growth obtained

proline for the same enzyme systems which convert these amino acids t other amino acids The hydroxyproline is less readily converted and so may act as a competitive inhibitor Because other amino acids are not as closely related structurally as these two they do not compete to the same extent However, if the medium is adequate with respect to all amino acids the need for action of these enzymes which convert one amino acid to another disappears, and *growth then would be expected to be less inhibited by* the presence of hydroxyproline

Summary

Thirteen species of fungi (or yeast) pathogenic for man have been grown

fungi and yeasts The amount and character of growth depends not only on the species used for inoculation but also on which amino acid is supplied as a source of nitrogen The amount of subsurface growth appears in general to

PUBLICATIONS

OF

THE NEW YORK ACADEMY OF SCIENCES

(LYCEUM OF NATURAL HISTORY, 1817-1876)

(1) The ANNALS (octavo series), established in 1823, contain the scientific contributions and reports of researches, together with the records of meetings of the Academy. The articles which comprise each volume are printed separately each in its own cover, and are distributed immediately upon publication. The price of the separate articles depends upon their length and the number of illustrations, and may be ascertained upon application to the Executive Director of the Academy.

(2) The TRANSACTIONS, re-established as Series II in 1938, contain extended abstracts of papers presented before the regular sectional meetings of the Academy and other matters of general interest to Members, and are published monthly from November to June, inclusive.

Current numbers of the ANNALS and TRANSACTIONS are sent free to all Members of the Academy desiring them.

(3) The SPECIAL PUBLICATIONS, established in 1939, are issued at irregular intervals as cloth bound volumes. The price of each volume will be advertised at time of issue.

(4) The MEMOIRS (quarto series), established in 1895, are issued at irregular intervals. It is intended that each volume shall be devoted to monographs relating to some particular department of Science. Volume I, Part 1, is devoted to Astronomical Memoirs, Volume II to Zoological Memoirs. No more parts of the Memoirs have been published to date. The price is one dollar per part.

(5) THE SCIENTIFIC SURVEY OF PUERTO RICO AND THE VIRGIN ISLANDS (octavo series), established in 1919, gives the detailed reports of the anthropological, botanical, geological, paleontological, zoological, and meteorological surveys of these islands.

Subscript[illegible]

EXECUTIVE DIRECTOR
The New York Academy of Sciences
2 East Sixty-third Street
New York 21, N. Y.